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Methods for the Translocation of the Yellow Lampmussel (*Lampsilis cariosa*) and the Tidewater Mucket (*Leptodea ochracea*) in the Fort Halifax Dam Impoundment of the Sebasticok River, Maine

Jennifer Elaine Kurth

METHODS FOR THE TRANSLOCATION OF THE YELLOW LAMPMUSSEL (*Lampsilis cariosa*) AND THE TIDEWATER MUCKET (*Leptodea ochracea*) IN THE FORT HALIFAX DAM IMPOUNDMENT OF THE SEBASTICOOK RIVER, MAINE

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By Jennifer E. Kurth

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An Abstract of the Thesis Presented
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Translocation from areas where habitat alterations are proposed can be an important mussel conservation tool. Pending removal of the Fort Halifax dam on the Sebasticook River in Maine potentially would result in extensive mortality of two state-listed threatened species of mussels, yellow lampmussels (*Lampsilis cariosa*) and tidewater muckets (*Leptodea ochracea*), which occur in the impoundment above the dam. My study assessed populations of these two species in the impoundment, and determined the effects of within- and between-waterbody translocations on survival. I conducted a qualitative survey of the Fort Halifax dam impoundment in 2004 to determine locations of these two species and a quantitative survey near the upper end of the impoundment in 2005 where the greatest numbers of these species occur. Estimated densities in survey plots were 0.05-1.1/m^2 for yellow lampmussels and 0.0-0.41/m^2 for tidewater muckets.

In a 2004 pilot study, I translocated a co-occurring common species, eastern lampmussel (*Lampsilis radiata radiata*), within the impoundment and to two other sites in the watershed, Unity Pond and Sandy Stream. Recapture rates for 2005-2006 were 34-

83% (0-9% mortality). As part of this effort, I used Passive Integrated Transponder (PIT) tags to track translocated mussels to assess the feasibility of this monitoring tool. Numbers of recaptured mussels differed among study sites; however, at all sites I found more tagged mussels with PIT pack searches with visual confirmation (72-80%) than with visual searches alone (30-47%). PIT tags offer improved recapture of translocated mussels and increased accuracy of post-translocation monitoring. I repeated the experiment in 2005 with yellow lampmussels and tidewater muckets. I recaptured 57-90% of yellow lampmussels (0-7% mortality) and 30-86% of tidewater muckets (4-6% mortality) using PIT pack searches with visual confirmation.

In Sandy Stream, sediment is redistributed annually with high late winter-early spring flows, which carry debris and stream-dwelling organisms downstream toward Unity Pond. I found 71% of recaptured eastern lampmussels >100 m from their October 2004 locations, and two yellow lampmussels and four tidewater muckets were 30-100 m downstream from their August 2005 locations. Yellow lampmussels and tidewater muckets in Sandy Stream were also significantly smaller than those found in the Sebasticook River. Although tidewater muckets and yellow lampmussels occur in this stream, the unstable stream bottom and high muskrat predation potentially threaten their survival, making this site unsuitable for translocating mussels from the Sebasticook River.

I found greatest densities of yellow lampmussels and tidewater muckets in boulder and cobble substrate in the upper 1.5 km of the impoundment. This area is least likely to be reconfigured following dam removal; the channel should be stable during dewatering and may be a refuge for all mussel species. Mussels in this section could then

repopulate the newly formed channel once it stabilizes in the middle of the impoundment. As long as care is taken to protect mussels during dewatering by translocating exposed mussels to the stable channel in the upper end of the impoundment, restoration of lotic habitat throughout the formerly impounded area will benefit yellow lampmussels and tidewater muckets in the long-term.

ACKNOWLEDGEMENTS

I would like to acknowledge the sources of funding for this project: the Maine Department of Inland Fisheries and Wildlife via the State Wildlife Grants Program and the Endangered and Nongame Wildlife Fund, the USGS-State Partnership Program, the USGS Maine Cooperative Fish and Wildlife Research Unit, the Maine Outdoor Heritage Fund, and the University of Maine Department of Wildlife Ecology. I would like to thank all the people who provided invaluable assistance and advice: Beth Swartz and Keel Kemper at MDIFW, Neil Greenberg at University of Maine Aquaculture Research Center, Ethan Nedaeu, Sean Werle, Christopher Rigaud—University of Maine Dive Safety Officer, the MDIFW Warden Service Dive Team, Gayle Zydlewski, Stephen Kneeland, Cory Stearns, Corey Gardner, and Erin Simmons. I would also like to thank Mark Hove at the University of Minnesota for introducing me to the wonderful world of freshwater mussels. I would especially like to thank my field assistants, Carolyn Currier and Rebecca Clark, for all their hard work. The members of my committee—Joseph Zydlewski and Michael Kinnison—provided invaluable advice on experiment design, data analysis and feedback on earlier drafts of the thesis. Most of all, I would like to thank my thesis advisors, Cynthia S. Loftin and Judith M. Rhymer, for countless hours of encouragement and advice.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

SURVEYS TO DETERMINE DISTRIBUTIONS AND DENSITIES OF TWO STATE-LISTED THREATENED FRESHWATER MUSSELS (YELLOW LAMPMUSSEL AND TIDEWATER MUCKET) IN THE FORT HALIFAX DAM IMPOUNDMENT OF THE SEBASTICOOK RIVER, MAINE

Introduction

North America has the greatest freshwater mussel biodiversity in the world, with nearly 300 species in the continental United States (Turgeon et al. 1988). More than 70% of these species now are considered "endangered", "threatened", or of "special concern", primarily due to water quality issues and habitat modifications such as channelization, dredging, dams, and impoundments (Williams et al. 1992). Mussels of the order Unionoida are unique in that they are obligate parasites of fish hosts during their larval (glochidial) stage. Glochidia encyst in the tissues of the fish host soon after attachment, metamorphose into juvenile mussels while attached to the fish host, then drop off to the stream bottom wherever the host is located at that stage of development. Thus, any impediment to fish dispersal, such as dams, affects freshwater mussel dispersal and the distribution of mussel populations in rivers and streams.

Dams and dam removal

Dams alter the physical, chemical, and biological environments of streams, both upstream and downstream, altering 30-60% of the mussel fauna (species composition and abundance), primarily by disrupting the reproductive cycle by eliminating fish host species (Williams et al. 1992). Dams trap sediments and debris moving downstream. These sediments may contain large quantities of contaminants or nutrients (Stanley and

Doyle 2003). Increasing nutrients can create eutrophic conditions in the impoundment that are not tolerated by most mussel species (Miller et al. 1992, Blalock and Sickel 1996). Turbidity may make mantle lures or other host attracting strategies less efficient for attracting fish hosts (Haag and Warren 1998). Fine sediments may be unsuitable substrate for some mussel species and become anaerobic during low discharge periods (Blalock and Sickel 1996). Impoundments lead to a decrease in mussel species richness and an increase in species that are more tolerant of siltation and are not host specific (Miller et al. 1992, Blalock and Sickel 1996).

The Association of State Dam Safety Officers estimates that by 2020, 80 % of the over 76,000 dams in the United States that are > 2 m high will require repair, replacement, or removal (Shuman 1995). Dam removal often is less expensive than repair or replacement, especially for marginally productive hydroelectric dams (Shuman 1995). Fish passage installation to enable anadromous fish migration often is a requirement for dam relicensing, and the expense may exceed projected revenues from hydroelectric power generation (Stanley and Doyle 2003). This situation pertains to the Fort Halifax dam on the Sebasticook River in Winslow, ME, which is slated for removal in 2007. Dam removal is a relatively new occurrence, and studies that have examined effects of dam removal on mussels are rare (Sethi et al. 2004). Changes in sediment transport, floodplain dynamics, and river channel morphology are environmental concerns during dam removal. Dam removal potentially can restore river temperature dynamics, flow patterns for migratory fish, and flood dynamics, but instability of sediments in the former impoundment also can occur (Gregory et al. 2002). Downcutting into sediments can result from dam removal, producing a deeper, narrower channel in the

impoundment, and distributing sediment downstream of the former dam site. Most geomorphic adjustments occur 1-5 years after dam removal (Stanley et al. 2002, Doyle et al 2005). Within 1 year following dam removal, benthic macroinvertebrate communities change from lentic to lotic assemblages (Stanley et al. 2002, Pollard and Reed 2004). Recovery of freshwater mussel communities in the impoundment is slower to occur (Sethi et al. 2004). While dam removal and the dewatering and channel instability that follows can be detrimental to mussels in the short-term, restoration of free-flowing habitat and access to fish hosts will ultimately benefit mussels by improving the system as a whole (Stanley and Doyle 2003).

Mussel survey methods

Mussel surveys are conducted to determine species presence, richness or density, spatial distribution, population size estimates, age and size profiles, changes in populations over time, and suspected effects of alterations at selected sites (Strayer and Smith 2003). Qualitative and quantitative surveys are useful methods of surveying freshwater mussels, and each has advantages and disadvantages (Strayer and Smith 2003). The method used is determined by the goals of the survey and the time available to complete the survey.

Qualitative surveys, also called timed searches or informal sampling, involve visual searches of a selected area and often are used to determine species presence and distributions (Strayer and Smith 2003). Qualitative surveys may overestimate population sizes of large species and underestimate those of buried or small species (Miller and Payne 1993, Miller et al. 1993, Strayer et al. 1993, Vaughn et al. 1995, Hornbach and Deneka 1996, Strayer et al. 1996, Obermeyer 1998).

In quantitative surveys, selected areas are intensively sampled to estimate species richness, unbiased estimates of relative abundance, or density, age and size profiles, and changes in population size over time (Miller and Payne 1988, Strayer and Smith 2003). Quantitative sampling can underestimate density of rare species and the total number of species (Miller and Payne 1993, Miller et al. 1993, Strayer et al. 1993, Vaughn et al. 1995, Hornbach and Deneka 1996, Strayer et al. 1996, Obermeyer 1998).

Often both qualitative and quantitative methods are used, with a preliminary qualitative survey to determine species distributions and a subsequent quantitative survey to determine the desired population parameters (Payne et al. 1995, Obermeyer 1998, Villella and Smith 2005).

The Fort Halifax dam and the Sebasticook River

The Sebasticook River is the largest tributary of the Kennebec River, entering the mainstem ~26 km upstream of Merrymeeting Bay. The mainstem of the Sebasticook is 45 km long and is impounded in many sections by hydroelectric dams. The Fort Halifax dam is located in Winslow, Maine, and is situated 427 m upstream of the Sebasticook River and Kennebec River confluence. Constructed in 1907-1908, the dam impoundment (1.4 km^2) extends ~8.4 km upstream to the Benton Falls dam. The dam's owner, FPL Energy Maine Hydro LLC (FPL Energy), is seeking to partially remove the dam. A 1987 agreement with the state required installation of fish passages at several dams, including Fort Halifax; FPL Energy has determined the cost of fish passages to be prohibitive and has opted to partially remove the dam in lieu of installing fish passages (Richter 2003). The pending removal of the Fort Halifax dam on the Sebasticook River will affect mussels both directly through habitat change and indirectly through fish host dispersal.

The Fort Halifax dam removal will dewater the impoundment, potentially causing extensive mortality of two state-listed, threatened species of mussels, the yellow lampmussel (*Lampsilis cariosa*) and tidewater mucket (*Leptodea ochracea*) found in the impoundment.

Status of yellow lampmussels and tidewater muckets

The yellow lampmussel has been considered for federal listing because it is believed to be declining throughout its range. It is also a species of special concern in Canada (Davis et al. 2004) and is listed as endangered (EN A1c) by the World Conservation Union (IUCN 1994) due to reduction in population size of at least 90% and a decline in area of occupancy, extent of occurrence and quality of habitat (Bogan 1996a). The tidewater mucket is listed as a species of special concern nationally and is also declining throughout its range. It is considered Near Threatened (NT) by the World Conservation Union (IUCN 1994, Bogan 1996b).

Both yellow lampmussels and tidewater muckets are Atlantic Slope species found historically from Georgia to New Brunswick (Nedeau et al. 2000). Yellow lampmussels are found in few watersheds in Maine (Sebasticook, St. George, middle Penobscot, and Passadumkeag River systems), and populations at these sites are considered healthy, because they are reproducing (Nedeau et al. 2000, Wick 2006). The largest populations of tidewater muckets in Maine are in the lower Kennebec and Penobscot River drainages (Nedeau et al. 2000). Both yellow lampmussels and tidewater muckets are found in a variety of substrates, including silt, sand, gravel, and cobble. Yellow lampmussels are found in medium to large rivers, but also occur in ponds, streams and impoundments; tidewater muckets are found primarily in coastal lakes, ponds, and slow moving rivers,

including impoundments (Nedeau et al. 2000). Because populations of these two species in Maine are reproducing and are relatively undisturbed compared to elsewhere in their range, Maine populations could represent a stronghold for these species (Nedeau et al. 2000).

Yellow lampmussels and tidewater muckets were found in the Sebasticook River Fort Halifax dam impoundment during a 1995 statewide mussel survey (Beth Swartz, Maine Department of Inland Fisheries and Wildlife (MDIFW) personal communication). Eastern lampmussels (*Lampsilis radiata radiata*) and eastern elliptio (*Elliptio complanata*) were common, but only two live yellow lampmussels, five yellow lampmussels shells and two tidewater muckets shells were found (Beth Swartz, MDIFW, personal communication). As part of the Incidental Take Plan (ITP) for the Fort Halifax dam removal, FPL Energy is required to translocate yellow lampmussels and tidewater muckets exposed during dewatering. Therefore, accurate assessments of yellow lampmussel and tidewater mucket densities and distributions in the impoundment are vital for optimizing this process. Since 1995, there have been two qualitative surveys of these species in the Fort Halifax impoundment. The first followed a drawdown in 1998, and 1,236 dead tidewater muckets and 251 dead yellow lampmussels were discovered primarily at the upper end of the impoundment within 1.5 km of the Benton Falls dam (Hanson 1998). In June 2003, FPL Energy conducted the second qualitative survey of the impoundment over a 2-day period as part of the ITP, using wading, snorkeling, and divers on dive planes pulled behind a boat. Based on that survey, the population of yellow lampmussels in the upper impoundment was estimated to be in the hundreds, but only ten tidewater muckets were found with wading surveys near the Fort Halifax dam.

Wick (2006) quantitatively surveyed an area of the upper impoundment with sandy substrate and estimated densities of yellow lampmussels of ~0.75/m^2 and tidewater muckets of ~0.25/m^2.

The objectives of my study were to perform qualitative and quantitative surveys of the Fort Halifax dam impoundment to more accurately determine distributions, densities, and population size structures of yellow lampmussels and tidewater muckets to assist efforts to conserve these two species when the dam is removed. The approach taken was a preliminary qualitative survey of the Fort Halifax impoundment to determine areas with apparent concentrations of mussels, followed by quantitative surveys conducted in areas of the impoundment where mussels are most abundant.

Methods and Materials

Qualitative surveys of the impoundment

I conducted qualitative surveys in the Fort Halifax dam impoundment during July 2004 to locate the greatest population densities of yellow lampmussels and tidewater muckets. I used snorkeling to survey 28 sites throughout the impoundment during 8 days (Fig. 1.1). The surveyed areas were placed ~500 m apart in 60 m lengths along the shore of both sides of the impoundment to 10-20 m offshore to a depth of 1.5-2 m (Fig. 1.1). I counted yellow lampmussels and tidewater muckets, estimated numbers of other observed species, and described the substrate type (boulder, cobble, silt, sand) in the area surveyed.

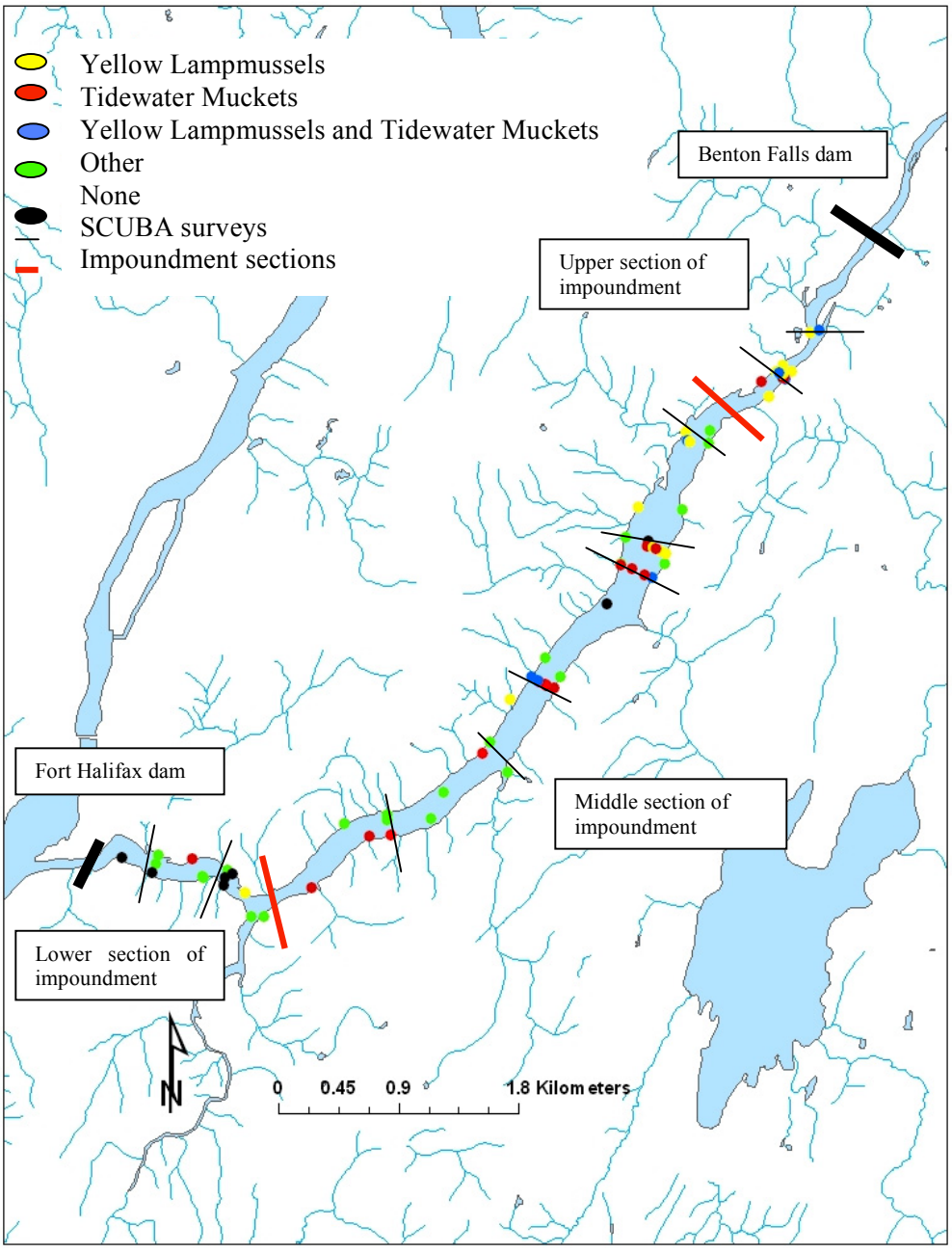


Figure 1.1. Qualitative survey sites for yellow lampmussels and tidewater muckets in the Fort Halifax dam impoundment of the Sebasticook River, Maine, 2004.

I surveyed ten additional areas of the impoundment (Fig. 1.1) over 2 days using snorkeling and diving with assistance from Maine Warden Service SCUBA divers. Pairs of divers swam along a weighted line placed bank to bank, searching for mussels within a meter of both sides of the weighted line, while snorkelers surveyed the shallow (<1.5-2 m depth) areas 10-20 m from shore and along 15-20 meters of shoreline on both sides of the rope. Sites were approximately 0.8 km apart. As with the above survey, numbers of yellow lampmussels and tidewater muckets observed, as well as estimates of the numbers of other species encountered, and the substrate type in the area surveyed were recorded in these surveys.

Quantitative surveys of the impoundment

Systematic sampling with multiple random starts is a probability-based sampling method used to determine mussel species age/size profiles, density, and population size estimates, as well as variation of the estimates (Strayer and Smith 2003). Sampling units are selected at regular distances from a random starting point, and each unit that follows from the random starting point is part of the same systematic sample. Multiple random starts allow for multiple systematic samples and are needed for variance calculations of population size and density estimates (Strayer and Smith 2003). I conducted quantitative surveys using systematic sampling with multiple random starts during July-August 2005. I used bank-to-bank snorkeling or SCUBA to survey areas in the upper end of the impoundment (within 1.5 km of the Benton Falls dam) where yellow lampmussels and tidewater muckets were determined to be most abundant in the 2004 qualitative surveys (Fig. 1.2). Quantitative surveys were conducted in 2 phases: snorkel surveys in shallow water (<1.5m) and SCUBA surveys in deep water (>1.5 m).

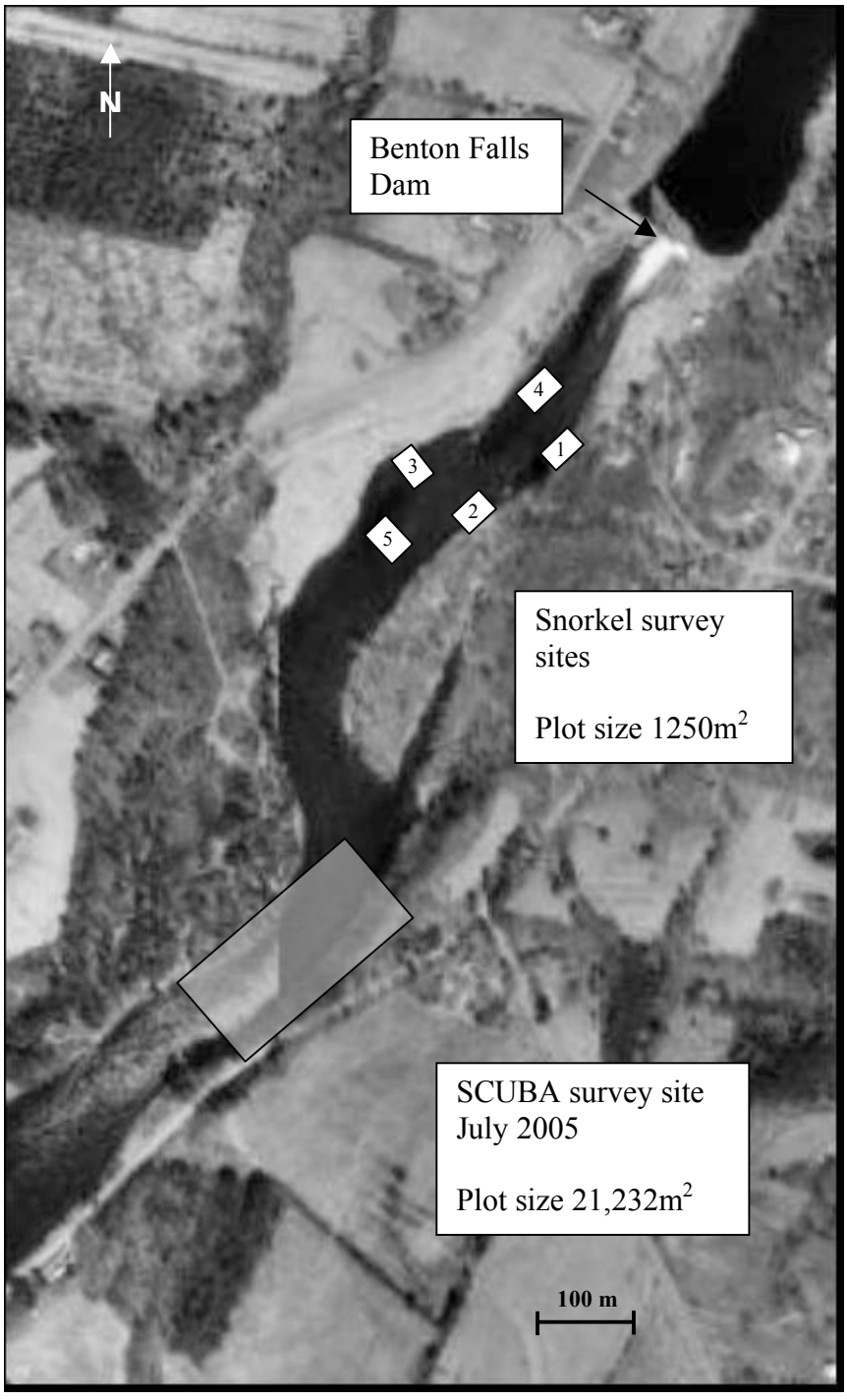


Figure 1.2. Quantitative survey sites for yellow lampmussels and tidewater muckets in the Fort Halifax dam impoundment of the Sebasticook River, Maine, 2005. Plots not shown to scale.

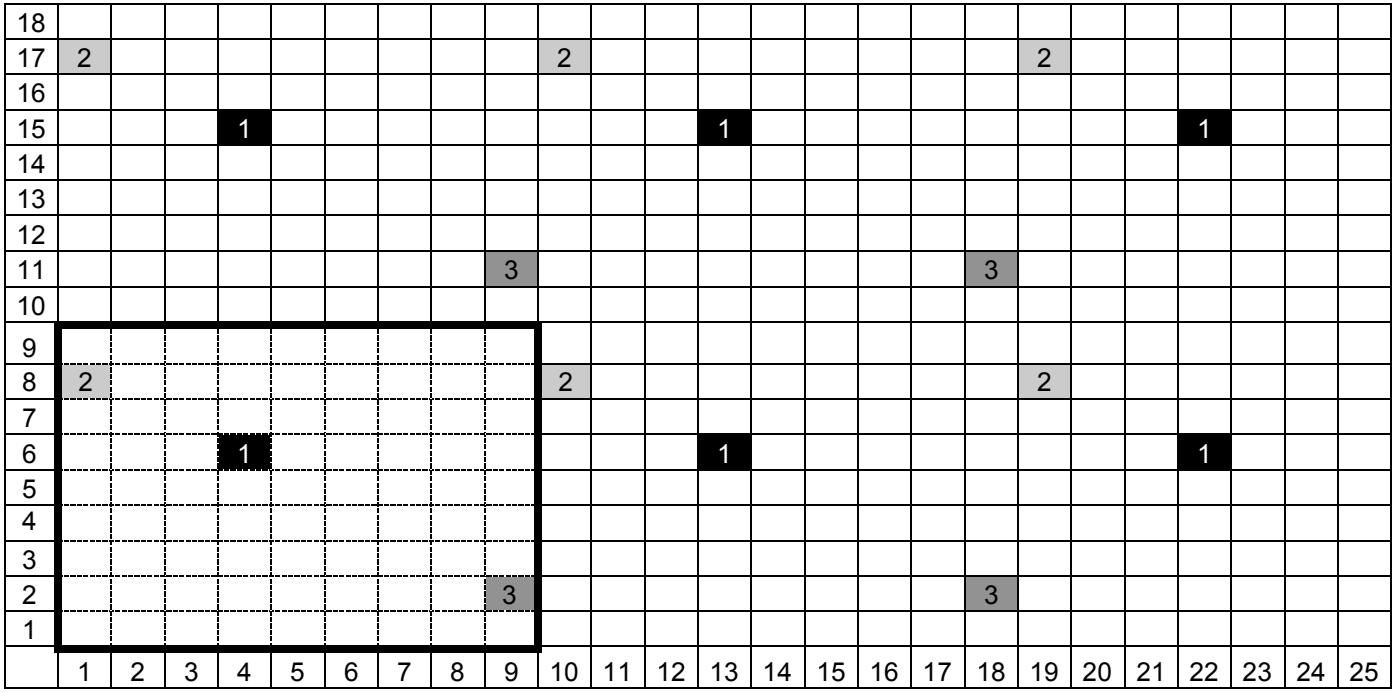


Figure 1.3. Example of systematic sampling design used for quantitative snorkel surveys. Three random coordinates were generated to serve as starting locations within the 9m by 9m area (defined by heavy border). Additional plots were selected at 9 m intervals. All plots that originate from a random starting coordinate are part of one systematic sample as shown by differing shades of black and gray and numbers.

For snorkel surveys, I delineated 5 areas (each 1250 m^2) in the upper impoundment with 50 m weighted rope placed along both shores. A 25 m weighted line marked at one-meter intervals was moved upriver along the marked 50 m lines to ensure appropriate placement of the quadrats. My sampling quadrats were 9 m apart, and I used 3 random starts (distances from the starting point) chosen by a random number generator (Fig. 1.3). I initiated snorkel surveys in one of the survey areas (area 3, Fig. 1.2) in August 2004, but the remainder of the survey was postponed until 2005 due to high water. I surveyed areas 1-4 (Fig. 1.2) in July 2005, but I found few tidewater muckets, so I repeated snorkel surveys at areas 1 and 3 and added area 5 in August 2005. I measured all yellow lampmussels and tidewater muckets for length, width, and thickness (to the nearest mm), replaced them in their original locations, and noted numbers of other species in each plot.

For the SCUBA survey, I placed weighted lines marked in one-meter intervals approximately 500 m downstream, where water was deeper (up to 4 m). The lines were placed on both sides of the impoundment and from bank to bank, to delineate an area of 21,232 m^2 (Fig. 1.2). In the first 2 transects, I placed the 3 random starts 24 meters apart so that there were 4 quadrats sampled per transect. Two SCUBA divers searched the quadrats in the first 2 transects. After observing that mussels were dispersed across the river bottom and few would be encountered at this quadrat density, I doubled the number of quadrats sampled per transect to 8, and the distance between the quadrats in each 24 m random start area was randomly selected to be 6, 12, or 18 m apart, alternating among

transects. The divers surveyed 229 1-m^2 plots and measured all yellow lampmussels and tidewater muckets before returning them to their original locations.

Population estimates calculated from quantitative surveys

I estimated population sizes separately for snorkel and SCUBA surveys. I determined distance between sampling units (d) for the quantitative surveys as

$$d = \sqrt{\frac{(L \cdot W)}{n/k}},$$

where L and W are the length and width of the sample area, n = the number of plots to be sampled, and k = the number of random starts (Strayer and Smith 2003). The estimate of mussel population size ($\hat{T}$) is the average count per systematic sample multiplied by the number of possible systematic samples (M),

$$\hat{T} = M\left[\frac{\sum_{i=1}^{m} x_i}{m}\right],$$

where m = the number of systematic samples. The variance for population size is calculated as,

$$\hat{\text{var}}(\hat{T}) = \frac{M(M-m)}{m} \cdot \frac{\sum_{i=1}^{m}(x_i - \bar{x})^2}{m-1},$$

I divided the population total by the area of the survey site to estimate population densities. The variance of the density estimate is the variance of the population size ($\hat{T}$) divided by area squared (Strayer and Smith 2003). I calculated 90% confidence intervals with a logarithmic transformation of the estimate and a delta-method approximation of variance based on Seber (1982):

$$\exp\left[\ln(\hat{T}) \pm t_{\alpha/2,df}\sqrt{\frac{\hat{\text{var}}(\hat{T})}{\hat{T}^2}}\right].$$

Results

Qualitative surveys of impoundment

I partitioned the impoundment into three sections (Fig. 1.1) based on substrate composition recorded by SCUBA divers. Substrate in the upper 1.5 kilometers of the impoundment consists primarily of boulder and cobble with maximum depths of 3.5m; the greatest number of yellow lampmussels and tidewater muckets were found in this section (Table 1.1). Mussels in this section were surveyed in 2 bank-to-bank combined SCUBA and snorkel survey transects and 7 snorkel-only surveys along the shoreline.

Table 1.1. Locations of yellow lampmussels and tidewater muckets in the Fort Halifax dam impoundment of the Sebasticook River, Maine, based on 2004 qualitative surveys.

Section*	**Substrate**	**# of yellow lampmussels**	**# of tidewater muckets**
Upper 1.5 km of impoundment	Boulder and cobble	97	40
Middle section of impoundment (~5.5 km long)	Silt/mud flat	21	31
Lower 1.5 km of impoundment from China Lake Outlet to Fort Halifax dam	Silt/sand/steep sides	2	1

*sections indicated on Figure 1.1.

The middle section of the impoundment (~5.5 km in length) consists of fine silt sediments and mud flats, with occasional patches of boulder/cobble. The impoundment varies in width (200-400m) in this section, with vast shallow mudflats (1-2m depth) that often are more than half the width of the impoundment. Depths in the original channel (before dam construction) range 4-7m. Although tidewater muckets and yellow lampmussels were observed in this region of the impoundment, they were less frequently encountered than in the upper impoundment section (Table 1.1). This section was surveyed in 6 bank-to-bank combined SCUBA and snorkel survey transects and 15 snorkel-only surveys along the shoreline.

The lower 1.5 kilometers from the Fort Halifax dam to the inlet from China Lake is characterized by steep, rock ledge composed of boulder and cobble and maximum impoundment water depth (9 m). Two yellow lampmussels and 1 tidewater mucket were found in 2 bank-to-bank combined SCUBA and snorkel survey transects and 6 snorkel-only surveys along the shoreline (Table 1.1).

Quantitative survey of the impoundment

In the SCUBA surveys, I found 81 yellow lampmussels and 42 tidewater muckets in the 21,232 m^2 survey area (Table 1.2). Results of the snorkel survey were more variable depending on site and time of surveys, with numbers of yellow lampmussels ranging 3-60 and numbers of tidewater muckets ranging 0-24. In the snorkel surveys, I found yellow lampmussels and tidewater muckets in slightly different habitats from each other, and there were differences between species in timing of emergence from the substrate.

Table 1.2. Population size and density estimates of yellow lampmussels and tidewater muckets in the Fort Halifax dam impoundment of the Sebasticook River, Maine, based on 2004-2005 quantitative surveys. Area surveyed in sites 1-5 was 1250 m^2 each, whereas SCUBA survey area was 21,232 m^2.

			Yellow lampmussel				Tidewater mucket			
Site [a]	**Habitat/flow**	**Survey date**	**Number found**	**Population estimate**	**90% CI**	**Density estimate**	**Number found**	**Population estimate**	**90% CI**	**Density estimate**
1	Cobble/high flow with sandy patches/low flow near bank	7/5/05	15	320	119-860	0.256/m^2	0	0	-	0.000/m^2
		8/11/05	38	789	318-1959	0.632/m^2	13	256	10-6842	0.205/m^2
2	Cobble/high flow/shallow	7/6/05	3	64	12-332	0.051/m^2	0	0	-	0.000/m^2
3	Cobble/boulders/high flow with some deep pools	8/12/04	22	594	536-658	0.475/m^2	17	459	365-578	0.367/m^2
		7/7/05	35	746	601-926	0.597/m^2	1	21	0-2.2x10^{14}	0.017/m^2
		8/12/05	60	1280	776-2112	1.024/m^2	24	512	195-1342	0.410/m^2
4	Rocky ledge/high flow	7/8/05	7	149	99-224	0.119/m^2	3	64	8-537	0.051/m^2
5	Cobble/boulders/high flow/shallow	8/16/05	33	917	817-1029	0.734/m^2	3	64	0-24,288	0.051/m^2
SCUBA	Cobble/boulders/very low flow	7/11-12/05	81	6912	5811-8205	0.326/m^2	42	3936	2185-7091	0.185/m^2

[a] Site numbers refer to snorkel plot locations in Figure 1.2.

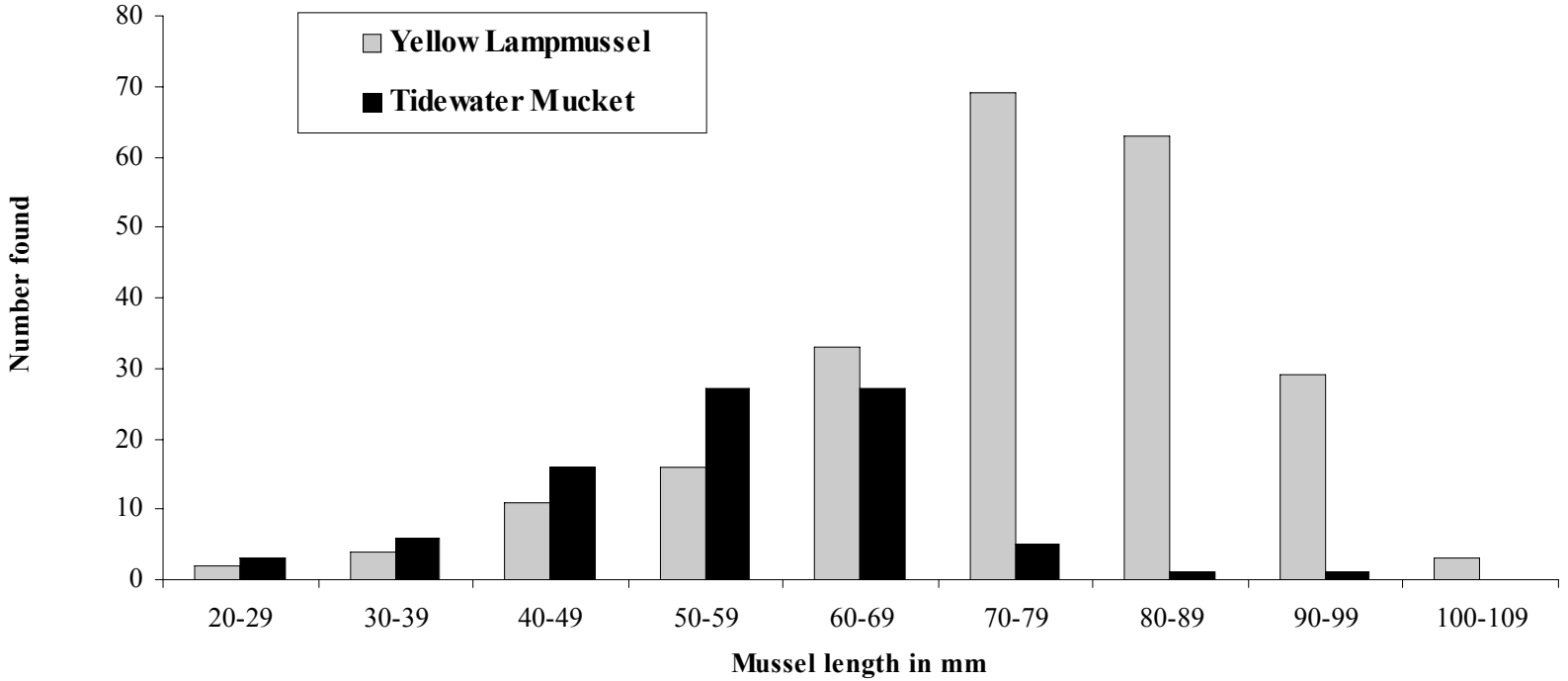


Figure 1.4. Size distribution yellow lampmussels and tidewater muckets found during quantitative surveys (snorkel and SCUBA) of the Fort Halifax dam impoundment of the Sebasticook River, Maine, 2005. Only August 2005 data were used for snorkel survey sites 1 and 3 to avoid duplication.

Yellow lampmussels emerged in July and were found in cobble substrates in areas with moderate to high flow rates. More yellow lampmussels were found when the sites were resurveyed in August. This increase may indicate that emergence occurs throughout the summer for this species. Tidewater muckets did not emerge until August and were found closer to shore in sandy substrate and low flow. In July, I found no tidewater muckets at sites 1 and 2, 1 at site 3, and 3 at site 4. In August I resurveyed sites 1 and 3 and found 13 tidewater muckets at site 1 and 24 at site 3. The increase in numbers found in August could also be due to more rigorous and accurate sampling, due to greater experience in sampling methods following the SCUBA surveys. The number of yellow lampmussels and tidewater muckets found per plot varied, with many plots containing none. Most plots that did contain yellow lampmussels and/or tidewater muckets had only 1or 2 of each species, with a few plots containing more. The largest number found in one m^2 plot was 9 yellow lampmussels and 2 tidewater muckets at site 1 in August 2005. The habitat surveyed by SCUBA was cobble/boulder with low flow conditions.

The number of yellow lampmussels and tidewater muckets found varied by habitat conditions and time of surveys, and, as a result, population and density estimates varied, as did the 90% confidence intervals of the population estimates. The size distributions of both mussel species indicated that individuals of all representative size categories were present except those < 20 mm in length (Fig. 1.4). Because the yellow lampmussel is generally larger than the tidewater mucket, there are more yellow lampmussels in the larger size categories. I found mussels < 20 mm in length during the snorkel surveys, but it was difficult to identify them to species at that size. As a result,

they are not included in the density and population estimates. Yellow lampmussels and tidewater muckets recorded in the snorkel surveys varied more in length than those found in the SCUBA surveys. For yellow lampmussels, the size of mussels found in the SCUBA surveys ranged 50-99 mm, whereas those found in the snorkel surveys were 20-109 mm. For tidewater muckets, the size of mussels recorded in the SCUBA (20-79 mm) and snorkel (20-99 mm) surveys were more similar.

Discussion

Distribution and Abundance of Yellow Lampmussels and Tidewater Muckets in the Impoundment

Yellow lampmussels and tidewater muckets were found throughout the impoundment in the qualitative surveys, but there were greater concentrations at the upper end of the Fort Halifax impoundment. Size class distributions of yellow lampmussels and tidewater muckets observed in the quantitative surveys (Fig. 1.4) suggest that their populations in the impoundment's upper end are actively recruiting. The quantitative SCUBA surveys were conducted within the area where mortality was high during the 1998 dewatering event, when 157 dead yellow lampmussels and 628 dead tidewater muckets were reported in this dewatered area (Hanson 1998). I estimate that there are 6912 (95% CI: 5811-8205) yellow lampmussels and 3936 (95% CI: 2185-7091) tidewater muckets in this area based on the results of the 2005 SCUBA survey, indicating that the populations persisted in spite of this mortality. No yellow lampmussels > 99 mm were found in this area, however, even though they can reach a maximum length of ~125mm (Nedeau et al. 2000). It is possible that this size class has not yet been replaced

following the 1998 dewatering. Tidewater muckets, on the other hand, do not usually grow to be more than ~85 mm in length (Nedeau et al. 2000), and I did find individuals near the maximum size in the SCUBA surveys. In the areas where I conducted quantitative snorkel surveys I found yellow lampmussels and tidewater muckets from all size classes >20 mm. I did not excavate quadrats during SCUBA and snorkeling surveys other than lifting rocks and fanning the top 2-5 cm of substrate, and mussels < 20 mm were not identified to species, so the proportion of individuals of each species in the <1 year age classes is unknown.

Yellow lampmussels and tidewater muckets occupied different substrate types in shallow water. I found yellow lampmussels in sites with higher flow rates and rocky/cobble substrates, whereas the thin-shelled tidewater muckets occurred in areas with lower flow rates and sandy/gravel substrates. During SCUBA surveys I found both species in deeper water with rocky/cobble substrates and lower flow. Tidewater muckets may be able to use this cobble substrate in deep, slow moving water, especially if they are behind large rocks, protected from high flows. Information about microhabitat preferences for these species is needed to predict where suitable microhabitat will exist for translocations in preparation for dam removal. Typically, yellow lampmussels are found in sand or gravel substrates in fast flowing sections of rivers (Nedeau et al. 2000, Davis et al. 2004). Tidewater muckets are typically found in a variety of substrates, including silt, sand, gravel, cobble, and occasionally clay (Bogan 1996b, Nedeau et al. 2000). This differs from my observation, suggesting that microhabitat use by these mussel species requires additional study.

Survey methods and timing

Selecting an appropriate mussel sampling protocol requires that the purpose of a survey be well defined. There must be clearly defined survey goals, adequate resources to conduct sampling, knowledge of the site characteristics, and information about the distribution of mussels at the site (Strayer and Smith 2003). Many locations should be sampled to accurately assess species distributions and abundances (Villella and Smith 2005). A full assessment of the population requires adequate search time for cryptic species and excavation to locate smaller individuals. In any mussel survey, there is a trade-off between general information about distribution versus specific information about demographics at a site. Using a combined qualitative and quantitative survey approach can provide information about both (Villella and Smith 2005).

Prior to this study there was little information available about distributions and densities of yellow lampmussels and tidewater muckets in the impoundment. Qualitative surveys provide spatial coverage and are relatively inexpensive to conduct (Villella and Smith 2005) and provided information about the areas of the impoundment with the greatest concentrations of these species. With the knowledge gained from qualitative surveys, I focused quantitative surveys on areas with higher densities of yellow lampmussels and tidewater muckets. The quantitative surveys provided precision for the population density estimates. This combined approach enables more efficient sampling; survey effort is concentrated where mussel densities are high, and the population estimate includes an assessment of accuracy of that estimate (Villella and Smith 2005).

The proportion of mussels visible at the substrate surface may vary by locality, time of year, species, and gender (Amyot and Downing 1997). Smith et al. (2001)

detected only 31% of clubshells (*Pleurobema clava*) at the substrate surface, whereas 52% of northern riffleshells (*Epioblasma torulosa rangiana*; 80% females, 45% males) were visible. During my translocation study (Chapter 3), I found 29% of tidewater muckets and 23% of yellow lampmussels completely burrowed into the substrate in July at Unity Pond, but by August, all mussels were found at the substrate surface. In Sandy Stream, which is always much colder than the Sebasticook River and Unity Pond, 53% of tidewater muckets and 80% of yellow lampmussels were completely burrowed in the substrate regardless of month. In August, only 8% of tidewater muckets and 1% of yellow lampmussels were found burrowed in the Sebasticook River. This is similar to Wick's (2006) observations, that >90% of yellow lampmussels, tidewater muckets, and eastern lampmussels had burrowed to 10-15 cm at Sandy Stream by August, but only 26% had burrowed in the Sebasticook River impoundment, where water temperatures were warmer at that time. Additionally, smaller individuals often remain burrowed into the substrate until they reach ~50mm in length (Amyot and Downing 1991, Balfour and Smock 1995).

Survey timing can affect accuracy of a population estimate. Day length and water temperature can cue vertical migration in freshwater mussels (Balfour and Smock 1995, Amyot and Downing 1997, Perles et al. 2003). Water temperature may cue mantle display behavior by yellow lampmussels with initiation at ~12^{o} C (Davis et al. 2004, Wick, 2006). The exact period of gravidity is unknown and may last into November (Davis et al. 2004). While tidewater muckets do not exhibit mantle display behavior, the gravid period is similar to that of yellow lampmussels (Wick 2006). Most tidewater muckets in my study area did not emerge from the substrate until August. When I

surveyed the sites in July, I found few tidewater muckets. When I resurveyed 2 sites in August, however, I found many more tidewater muckets at the substrate surface. The Incidental Take Plan (ITP) surveys were conducted in June; this survey date may have been before tidewater muckets emerged, resulting in low counts during the ITP survey. Yellow lampmussels were easier to detect, especially when females were exhibiting mantle displays, although males and non-brooding females may have been overlooked during cursory surveys.

Limitations of qualitative and quantitative surveys

Qualitative surveys were conducted over 10 days (8 for snorkel only and 2 for SCUBA plus snorkel). The results for both types of surveys were similar, however, so conducting the 2-day SCUBA plus snorkel surveys would have been adequate for learning the distributions of yellow lampmussels and tidewater muckets in the impoundment. The number of person hours needed to conduct the qualitative surveys was the same for the snorkel only and SCUBA plus snorkel surveys (160 h), so time to complete and manpower resources available should be a consideration in determining which to use. Even though the duration of the SCUBA plus snorkel surveys was similar to that of the ITP survey, the results differed. The ITP survey underestimated the abundance and distribution of yellow lampmussels and tidewater muckets throughout the impoundment. A more accurate assessment of the distribution of these species in the impoundment resulted from my more systematic, qualitative impoundment survey.

Because systematic sampling with multiple random starts is a probability-based sampling method, it is possible to determine mussel species density, population size estimates, and age/size profiles, as well as the variation of the estimate. This method

increases survey extent, and multiple random starts allow for multiple systematic samples, which are needed for variance calculations of population size and density estimates (Strayer and Smith 2003). If there is only one random start, it must be assumed that the mussels are distributed randomly in order to estimate variance (Strayer and Smith 2003). Because mussel distribution tends to be patchy, this would be an incorrect assumption (Downing and Downing 1992). I adjusted the distances between sample units during the SCUBA surveys, because the distance originally selected was too large and there was sufficient time to survey more plots. By increasing plot number, I was able to increase coverage of the survey area and calculate more precise estimates of mussel densities.

The visibility during the SCUBA surveys was less than that found during the snorkel surveys, due to greater depths, and silt that had accumulated in the low flow. The difference in the size of mussels found may be due to lower visibility, especially for the smaller size classes. Visibility also was impaired during the qualitative surveys in the middle section of the impoundment where the substrate was composed of fine silt that was easily disturbed. In situations of poor visibility it may be better to postpone the survey or excavate, remove, and sieve the substrate to collect any mussels that may be overlooked.

Implications for management

There were more yellow lampmussels and tidewater muckets found in the qualitative surveys of the impoundment, and they were more widely distributed, than were found during the 2-day ITP surveys. The quantitative surveys suggest that population sizes and distributions were underestimated by the ITP surveys. Recovery

efforts during the Fort Halifax dam removal should be focused at the upper end of the impoundment; although this area may not experience complete dewatering with the dam removal, the greatest concentration of yellow lampmussels and tidewater mussels exist in this section of the impoundment. The area where the snorkel quantitative surveys were conducted is least likely to be affected during dewatering, whereas the region of the impoundment where the SCUBA quantitative surveys were conducted will be affected during the dewatering (Richter 2003). The middle section of the impoundment with vast mud flats is likely to be dewatered with dam removal. While there were not many yellow lampmussels and tidewater muckets found in this area, there were tens of thousands of the more common species (eastern lampmussels, eastern elliptio, and eastern floaters (*Pyganodon cataracta*)) (see Appendix I), which will be stranded during dewatering. FPL Energy is not required to move these mussels, so there will be massive mortality of the common species when the impoundment is dewatered, and only mussels that are in the area where the new channel will form will survive the initial dewatering. They still may be buried, however, in redistributed sediments or dislodged and transported downstream in flow. The channel will be most unstable in the mud flats, and this area of the impoundment may be void of mussels until the channel stabilizes, possibly several years after the dam is removed.

The ITP proposes translocating mussels to Unity Pond and Sandy Stream, because the post-dam removal channel is expected to be unstable. While post-dam removal channel instability has been documented in other dam removals, it is dependent in part on the sediment fill in the impoundment (Pizzuto 2002, Stanley and Doyle 2003). In the lower section of the Fort Halifax dam impoundment where the substrate is composed

primarily of fine silt, channel instability is likely. In the upper section, where I found the greatest densities of yellow lampmussels and tidewater muckets, however, the substrate is composed almost entirely of boulders and cobble, which are more stable. The channel formed in this section during dewatering should be relatively stable. Survival of mussels exposed during dewatering may be greater if the mussels are moved to this more stable channel, rather than translocation to other waterbodies. However, care must be taken in placing translocated mussels, especially tidewater muckets, in suitable substrates or flow refugia, as water velocities will likely increase in this area following the dam removal. This area may be an upstream refuge from which mussels can repopulate the newly formed downstream channel once it stabilizes following dam removal.

Chapter 2

PIT TAGS INCREASE EFFECTIVENESS OF FRESHWATER MUSSEL RECAPTURES

Introduction

A goal in the National Strategy for the Conservation of Native Freshwater Mussels is to "develop, evaluate, and use the techniques necessary to hold and translocate large numbers of adult mussels" (National Native Mussel Conservation Committee 1997). Successful recovery of translocated mussels is essential for accurate assessment of translocation success. Previous studies of freshwater mussel translocation used visual searches to recover mussels with varied success (Layzer and Gordon 1993, Havlik 1995, Bolden and Brown 2002, Cope et al. 2003). Survival estimates of translocated mussels often are based on the number of mussels recaptured or found dead, and non-recaptured mussels are assumed to have emigrated from the study site (Dunn and Sietman 1997, Hamilton et al. 1997, Dunn et al. 2000). A review of 33 mussel translocation studies found a mean estimated survival rate of 51% (although mortality was not reported in 27% of the studies); the average recapture rate was 43% (range: 1–97%) (Cope and Waller 1995).

Passive Integrated Transponder (PIT) tags may be an effective tool for tracking translocated mussels to increase accuracy of survival estimates. PIT tags are electronic glass-encased microchips that are activated by an inductive coil. They can be attached to an organism internally or externally. The tag is passive until activated by a fixed or portable reader with an antenna. When activated, the tag transmits a unique code to the reader, identifying the individual organism (Gibbons and Andrews 2004). Tag longevity

is indefinite, because an internal power source is not needed. In aquatic systems, PIT tags have been used extensively to study fish passage past stationary antennae or readers (Zydlewski et al. 2001). Portable PIT tag systems are used in shallow waters to assess spatial distributions of local fish populations, fine-scale movements, and microhabitat preferences (Roussel et al. 2000, Hill et al. 2006). This mobile application is ideally suited to freshwater mussel translocation studies because mussel movements often occur over short distances.

Traditional mussel recapture methods depend on visual encounters and excavation to locate burrowed mussels. PIT tags may enhance mussel recapture at sites where visibility is poor (e.g., turbid water) or when mussels are burrowed in sediments. Reliability of any tagging method depends on tag retention. The tagging method selected for freshwater mussels depends on shell thickness and the type of habitat into which the tagged mussels will be placed. Internal tagging may be best for thicker-shelled species, whereas external PIT tag placement may be more appropriate for thin-shelled species. In a fast-flowing environment with a rocky substrate, an external PIT tag might be dislodged, whereas an internal PIT tag would be protected from abrasion.

We designed an experiment to evaluate the use of PIT tags to mark and track individual freshwater mussels as part of a larger study to determine the feasibility of translocations of 2 state-listed threatened mussel species (tidewater mucket, *Leptodea ochracea*, and yellow lampmussel, *Lampsilis cariosa*) in response to an impending dam removal. The objectives of our study were to evaluate internal and external PIT tagging methods, retention, and post-tagging survival in freshwater mussels and to determine the effectiveness of PIT tag technology for mussel recaptures. We used the relatively

common eastern lampmussel (*Lampsilis radiata radiata*) as a surrogate for the listed species to develop the method. We tested internal tagging methods for future use with thicker-shelled species (e.g., yellow lampmussel) and external attachment for use with thin-shelled species (e.g., tidewater mucket).

Methods and Materials

Internal PIT tagging: mantle separation

We used 2 methods to place internal PIT tags. For method 1 ("*mantle separation*"), we placed the mussels in sandy substrate, waited until they were actively siphoning and slightly gaped, and then inserted a micropipette tip between the valves to separate them by ~5 mm. We teased the mantle tissue away from the shell, and inserted the PIT tag (Digital Angel, South St. Paul, Minnesota) between the mantle and shell along the mid-ventral margin. We also marked all mussels externally with numbered bee tags (The Bee Works, Orillia, Ontario) cemented (GC Fuji I Glass Ionomer Luting Cement, Henry Schein, Melville, New York) to the posterior end of the left valve. We sealed the bee tags with Delton Light Curing Pit & Fissure Sealant (Henry Schein). Control mussels received only the numbered bee tags. We were able to tag ~20 mussels/h with this method. Most of our time was spent waiting for mussels to gape so we could insert the micropipette tip.

In October 2004, we collected eastern lampmussels (55–101 mm length; $n = 164$) from the impoundment that will be dewatered following the Fort Halifax dam removal in the Sebasticook River near Winslow, Maine. In November 2004 (24–35 d after capture), we partitioned the mussels into a control ($n = 40$) and 3 tag-type treatment groups: 23-mm tags ($n = 40$), 12-mm tags ($n = 44$), and 12-mm tags with an antimigration cap (a

plastic sleeve encasing one end of the 12-mm tag to encourage tissue adherence [Biomark, Boise, Idaho]; $n = 40$]). Each group consisted of mussels of all sizes (control: 55–99 mm length, 23-mm tags: 58–101 mm length, 12-mm tags: 58–99 mm length, 12-mm tags with cap: 58–96 mm length).

We maintained mussels in the Aquaculture Research Center (ARC), University of Maine, Orono, Maine, in three 2.44 m x 0.61 m x 0.30 m fiberglass tanks filled with sand (13 cm deep) and recirculating water. We divided the mussels in each group among 3 replicates (13–15 mussels/replicate) and distributed 1 replicate from each group in each tank.

We fed the mussels an algal diet (*Phaeodactylum tricornutum*, *Chaetocerus-B*., and *Nannochloropsis oculata;* Algae Spat Formula, Innovative Aquaculture Solutions, Inc., Vancouver, BC) 3 times/wk. During each feeding, we stopped water recirculation and applied 40-50 x 10^9 algal cells/tank (R. Mair, Virginia Polytechnic Institute, personal communication). To simulate changes in seasonal water temperature, we gradually reduced water temperature from 18 ^{o}C (October) to 10 ^{o}C (December) and maintained 10 ^{o}C until the following April, then gradually increased the temperature to 18 ^{o}C by June. We monitored the mussels for mortality 3 times/wk and examined them for tag retention in November 2004 and February, April, and June 2005.

Internal PIT tagging: mantle incision

We developed a 2nd internal PIT tagging method ("*mantle incision*") with techniques from the cultured pearl industry (H. Dan, Virginia Polytechnic Institute, personal communication). We implanted PIT tags by inserting a micropipette tip between the mussel valves to separate them by ~5 mm, making an incision with a scalpel

in the mid-ventral mantle tissue, inserting the tag between the mantle and the shell through the incision, and then removing the micropipette tip. All mussels were also externally marked with bee tags on the posterior end of the left valve. Inserting the tags took little time (20 mussels/h). Most of our time was spent waiting for mussels to gape, so we could insert the micropipette tip.

In June 2005, we collected 112 eastern lampmussels (43–101 mm length) from the Sebasticook River impoundment and randomly assigned the mussels into 3 groups consisting of a control (n = 27) and 2 tag-type treatment groups (23-mm tags: n = 43, 12-mm tags with an antimigration cap: n = 42) with 3 replicates/group (9–15 mussels/replicate), being careful to include mussels of all sizes in each group. We did not test the 12-mm tags without caps because of poor retention in the mantle-separation experiment.

We maintained tagged mussels in the ARC for 21 d to ensure tag retention and then placed 1 replicate from each group in sand in each of 3 enclosures (1 m × 2 m polyvinyl chloride [PVC] pipe and rebar frames covered in hardware cloth) in Unity Pond, Maine. Unity Pond is a 1039-ha lake connected to the Sebasticook River upstream of the Winslow mussel collection site. Unity Pond contains a natural population of eastern lampmussels, and thus, is suitable habitat for the species. Before placing the mussels in the enclosures, we reinserted rejected tags (n = 9). We examined the mussels to assess tag retention and survival 60 d (August 2005) and 371 d (June 2006) after tagging.

External PIT tagging

We tested the reliability of external PIT tag attachment and determined the probability of recapturing translocated PIT tagged mussels that were not confined to enclosures (as in the previous experiment). We placed external PIT tags on 238 eastern lampmussels (41–88 mm length) collected during September and October 2004 from various sites in Unity Pond (n = 90), Sandy Stream (a 1st-order, spring-fed stream that drains into Unity Pond; n = 88), and the Sebasticook River impoundment near Winslow (n = 60). We chose these water bodies because they had naturally occurring populations of eastern lampmussels and the 2 listed species and because, based on neutral markers, Sebasticook River and Sandy Stream populations of these mussels were genetically similar (Kelly 2004).

We tagged mussels by cementing a PIT tag to the posterior end of the right valve and a numbered bee tag to the posterior end of the left valve. After the first 30 tags (at Unity Pond), we completely encapsulated the PIT tag in dental cement to increase tag retention. We placed tagged mussels in water before the cement was fully cured (~5 min after application) to avoid overdrying and cracking of the cement. We tagged ~30 mussels/h with this method. Most of our time was spent waiting for the bee-tag sealant to dry. We used 23-mm tags at all sites. We also used some 12-mm tags at Sandy Stream and Unity Pond because of a limited supply of cement.

We compared survival of translocated mussels among within-water body, between-water body, and within-site (control) translocation treatments. We measured, tagged, and moved mussels to 1 m × 2 m plots or replaced them where they had been

found (Table 2.1). We marked the corners of the plots with stakes with flagging, and recorded global positioning system (GPS) locations for each plot and for each of the tagged mussels that were returned to their original location.

Table 2.1. Numbers of mussels tagged with Passive Integrated Transponder (PIT) tags in each translocation treatment during September and October 2004.

	Tagged and replaced (site control)	Moved within water body	Translocated from Sebasticook River
Sandy Stream	30	26	32
Unity Pond	30	30	29
Sebasticook River	30	30	–

We recaptured externally PIT tagged mussels with a mobile PIT detection unit (PIT pack). The PIT pack used Destron Fearing FS1001A DC-powered, full duplex transceivers and custom-designed portable antennas (Hill et al. 2006). When a PIT tag was within range of an antenna (~0.5 m), the tag emitted a 134.2 kHz (ISO standard frequency) radio frequency, which was transmitted back to the receiver for decoding. The antennas, enclosed in an airtight PVC wand and attached to the transceiver, consisted of several wraps of 12-18 gauge wire, with inductance values ranging 325-375 □H, and a set of capacitors (Hill et al. 2006). The capacitors were attached to an antenna lead cable from the transceiver, fixing the capacitance between 33 and 44 nF. The fixed capacitance was used within the transceiver in conjunction with the adjustable capacitance to tune the resonance frequency of the system to 134.2 kHz (Hill et al. 2006). We tuned the adjustable capacitor while antennas were submerged. We conducted all field experiments with the PIT pack tuned to phase 0-2%, signal 1-20%, and current 2.5-5.0 amps.

We searched the release sites for externally PIT tagged mussels ~30 d after tagging (October 2004) and visually confirmed "recaptures" with snorkeling. If the PIT tag reader registered a tag but no mussel was observed, we assumed the mussel had burrowed into the substrate. To minimize substrate disturbance, we did not excavate burrowed mussels preparing to overwinter. These data were not used in the calculations of recapture success, because the signals may have been from detached tags. During June-July 2005 (271-355 d post-tagging) and July-August 2006 (670-750 d after tagging) we searched again for PIT tagged mussels at the release sites beginning at the last location recorded with GPS during October 2004. In 2005, we conducted initial searches without the PIT pack to provide recapture percentages with visual searches only. We visually searched each site for 2 d. Approximately 1 wk later, we searched the sites using PIT pack searches with visual confirmation and excavation to confirm recaptures (3-4 d per site). In 2006 we repeated the PIT pack searches with visual confirmation (3 d per site). Water clarity was too poor to conduct visual searches in 2006. If the PIT pack detected a tagged mussel, but we did not see the mussel, we excavated the area within 0.5 m of the signal to 15-45 cm deep to determine if the signal was coming from a burrowed mussel or an unattached tag. If we found no tagged mussel after excavation, we assumed the tag had become detached.

We searched (with snorkeling and the PIT pack) the sites at Unity Pond and the Sebasticook River 4 times each to at least 3 m beyond the perimeter of the original study area to detect mussels that may have moved. We also searched the shorelines for valves from dead mussels. Extensive ice scouring and spring flooding substantially reconfigured the substrate at the Sandy Stream site, so in addition to searching the study

area plus 3 m beyond the perimeter, we also swept the antenna bank to bank downstream of the site for 200 m over a total of 3 d. We calculated recapture rates by dividing the number of mussels recaptured at each site by the number tagged.

Data Analysis

We used adjusted χ^2 for small sample sizes (Gotelli and Ellison 2004) for all analyses. We compared long-term tag retention among tag types and mussel mortality among treatments and controls for both mantle separation and mantle incision methods. We compared the percentages of recaptures using visual searches alone with the number of recaptures using PIT-pack searches with visual confirmation.

Results

Mussel retention of internal PIT tags in the laboratory (*mantle separation)*

Five percent of the PIT tags were rejected within 2 wk of internal placement via mantle separation. By 100 d after tagging, rejection had increased to 10% for 12-mm tags with caps, 12.5% for 23-mm tags, and 30% for 12-mm tags without caps. High mortality with this method was more troubling than the rejection rates. By 100 d after tagging, mortality rates were 3% for the control group (no tags), 10% for the group with 12-mm tags with caps, 25% for the group with 23-mm tags, and 27% for the group with 12-mm tags without caps. This mortality may have been caused by inexperience with the tagging procedures and mussel aquaculture husbandry (mortality in control mussels was 3% 100 d after tagging and 73% 244 d after tagging), so we discontinued using the 12-mm tags without caps, switched to the mantle-incision method, and retained the tagged mussels in field enclosures.

Long-term tag retention did not differ among tag types (adjusted $\chi^2 = 5.61$, $p = 0.691$, df = 8) or in mortality among the tag-type and control groups 100 d after tagging (adjusted $\chi^2 = 7.97$, $p = 0.716$, df = 11). We examined the condition of the PIT tags in all mussels that died over winter. By 90 d after tagging, all 12-mm PIT tags with caps were coated with nacre and attached to a valve. By 120 d after tagging, 23-mm and 12-mm PIT tags without caps that had not been rejected were similarly attached.

Mussel retention of internal PIT tags in field enclosures (*mantle incision*)

By 60 d after tagging (40 d after transport from the ARC to the Unity Pond enclosures), all mussels in the control and tag-type groups (mantle incision) were still alive (Table 2.2). One 23-mm tag was rejected after the mussels were placed in the enclosures; this rejected tag was not one of the tags that had been rejected and reinserted within the 2-wk post-tagging observation period. By June 2006 (371 d after tagging), 2 mussels in the enclosures had died (1 control, 1 with a 23-mm tag), and one 12-mm tag with cap was rejected. At 371 d after tagging, long-term tag retention did not differ among tag types (adjusted $\chi^2 = 4.26$, $p = 0.833$, df = 8), and mortality did not differ among control and tag-type groups (adjusted $\chi^2 = 3.72$, $p = 0.882$, df = 11) (Table 2).

Table 2.2. Percent mortality and % tag retention (60 d and 371 d after tagging using the mantle-incision method) of eastern lampmussels with internal Passive Integrated Transponder (PIT) tags in field enclosures in Unity Pond, Maine.

	60 d after tagging		371 d after tagging	
Treatment	% mortality	% tag retention	% mortality	% tag retention[a]
23-mm tag (n = 43)	0	98	2.5	97.5
12-mm tag with cap (n = 41)	0	100	0	97.4
Control (no tag) (n = 27)	0	–	4.3	–

[a] Includes mussels that died with retained tags

Retention of external PIT tags and recapture of mussels in the field

Overall, 93% of the recaptured tagged mussels retained the PIT tag (Table 2.3). Recapture rates with PIT-pack searches with visual confirmation exceeded recaptures from visual searches alone at all study sites during June and July 2005 (adjusted χ^2 =10.198, p = 0.0014, df = 1; Fig. 1). During June and July 2005 and July and August 2006, we used a combination of visual searches alone and PIT-pack searches with visual confirmations to recapture 77% of externally tagged mussels at Unity Pond and 80% of externally tagged mussels in the Sebasticook River. In Sandy Stream, where ice scouring and spring flooding reconfigured the substrate, we recovered only 25% of the tagged mussels. Ninety-five percent of the mussels we did recapture were found using PIT-pack searches with visual confirmation, and only 1 mussel was found using visual searches alone.

Table 2.3. Percent recapture, % mortality, and % tag retention of externally Passive Integrated Transponder (PIT)-tagged eastern lampmussels in translocation experiments within and between sites (~21 mo after tagging) in Maine.

Site[a]	Treatment	Number tagged	% recapture	% mortality[b]	% tag retention[c]
Unity Pond	Translocated from Sebasticook River impoundment	29	93.1	0	100
	Translocated within Unity Pond	32	74.2	0	78.3
	Site control (not moved)	30	63.3	0	89.5
Sebasticook River	Translocated within Sebasticook River impoundment	30	93.3	0	96.4
	Site control (not moved)	30	66.7	6.7	100
Total		151	78.0	1.3	93.2

[a] Sandy Stream data omitted because of winter ice scouring and spring flooding

[b] Percent mortality calculated only for recaptured mussels

[c] Retention calculated as % recaptured mussels retaining tag

In Sandy Stream, we found 71% of recaptured mussels >100 m from their October 2004 locations, whereas we found recovered mussels in Unity Pond and the Sebasticook River <2 m from their September–October 2004 locations. Seventeen (Unity Pond), 17 (Sebasticook River), and 3.5% (Sandy Stream) of the recaptured mussels found with the PIT pack were completely burrowed into the substrate (Fig. 2.1). We found most burrowed mussels within 6 cm of the sediment surface. However, the PIT pack detected 1 tagged (23-mm tag) living mussel burrowed 45 cm into the substrate and 3 tagged dead mussels 20 to 30 cm below the substrate surface in Sandy Stream. We also found 1 dead mussel with a PIT tag during shore sweeps at the Sebasticook River site.

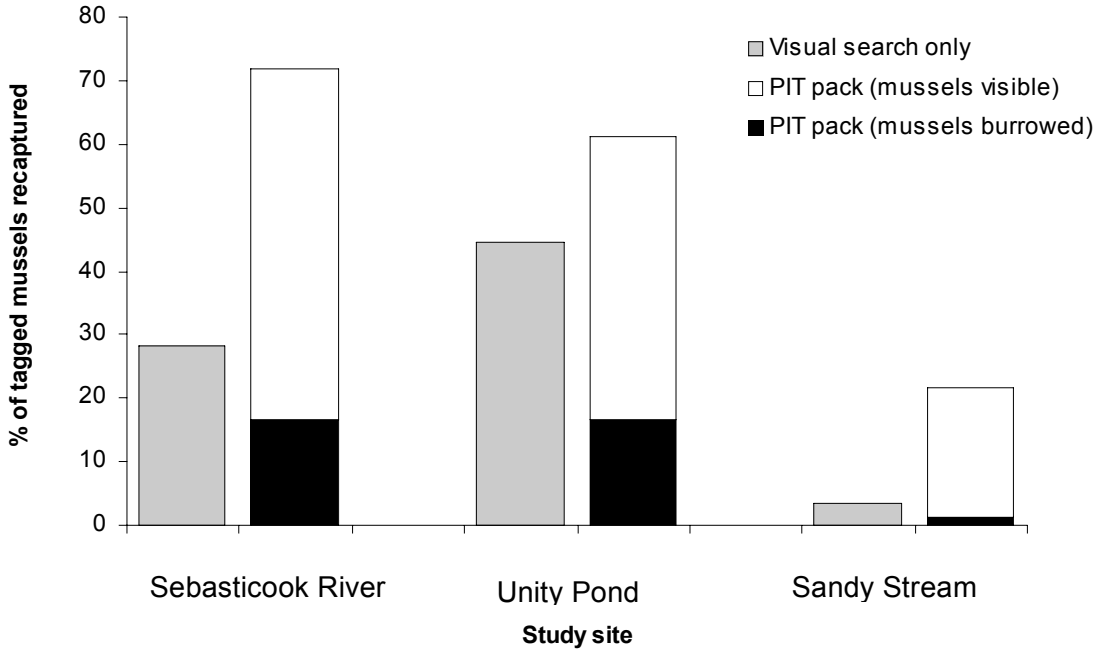


Figure 2.1. Recapture percentages of mussels externally tagged with Passive Integrated Transponder (PIT) tags for different recapture methods during June and July 2005.

Discussion

Tagging methods

Low mortality (<2%), high tag retention (97%), and evidence that tags had fused to the shell 3 to 4 mo after tagging suggest that internal PIT tagging using the mantle-incision method may be a viable method of tagging thick-shelled freshwater mussel species that can be pried open for tag insertion without damaging the shell. Long-term survival of captive freshwater mussels is low (Patterson et al. 1997, 1999, Nichols and Garling 2002a), and high mortality of captive mussels in our study (73–93% 255 d after tagging) might be attributed to inadequate nutrition, winter water temperatures in the ARC that exceeded temperatures at the mussel collection sites, and physiological stresses experienced by captive mussels that were gravid when captured. The low mortality of mussels tagged with the mantle-incision method and placed in the enclosures at Unity Pond supports this assertion. We strongly recommend field trials rather than aquaculture experiments for testing methods intended for use in the field to remove uncertainty of the effects of captivity on mussel survival.

External PIT-tag retention also was high (93%) when the PIT tag was completely encapsulated in cement and the mussel was placed in water within 5 min of cementing. However, retention was more variable with external tagging than with internal tagging methods, and ranged from 78 to 100% at the Unity Pond site 9 mo after tagging. We attribute low retention to incomplete coverage with cement. Retention of tags completely encapsulated with cement ranged from 89.5 to 100%. We observed evidence of some cement loss from recaptured mussels; occasional reapplication of cement will ensure long-term retention of external PIT tags. Internal tag placement via mantle incision is a

viable alternative to external attachment in environments where tag loss from abrasion is likely.

Previous studies assessed external freshwater mussels tagging methods with visual searches to relocate mussels marked with numbered tags (Lemarié et al. 2000) or coded wire tags inserted into mussels held in suspended pocket nets (Layzer and Heinricher 2004). Both of these tagging methods resulted in higher tag retention than in our study, but mussels tagged using these methods can be detected only with visual searches. However, PIT tags provide an alternative tool for finding mussels, and this method is especially useful for long-term monitoring or where visual searches are impractical or time consuming.

Mussel recapture efficiency

The proportion of mussels visible at the substrate surface may vary by locality, time of year, species, and gender. Smith et al. (2001) detected only 31% of clubshells (*Pleurobema clava*) at the substrate surface, whereas 52% of northern riffleshells (*Epioblasma torulosa rangiana*; 80% females, 45% males) were visible. Wick (2006) observed that >90% of eastern lampmussels had burrowed to 10 to 15 cm at Sandy Stream by August, but only 26% had burrowed in the Sebasticook River impoundment at that time.

We found burrowed mussels and mussels that would have been overlooked had the sites been searched only visually because the water was turbid. For example, water clarity in Unity Pond was routinely poor, and only 47% of tagged mussels were recaptured visually, whereas 72% of tagged mussels were recaptured with the PIT pack and visual confirmation. In the Sebasticook River, where the visibility was compromised

by silt covering the mussels, the recaptures with the PIT pack and visual confirmation (80%) were more than double that of the visual searches alone (29%). Initially, PIT tags also provided a visual cue of tagged mussels in clear water, but after several months in the water, the cement was stained or covered with algae and indistinguishable from the shell. When first applied the white cement might provide a visual cue to predators, but only 1 shell was found in a shoreline midden in our study. Tinting the cement a dark color might eliminate this possible problem.

Low recaptures in Sandy Stream probably were caused by extensive downstream displacement of mussels in late winter and early spring when ice scour and high water flows during snowmelt reconfigured the stream bottom. The low recapture rates of PIT-tagged mussels at this site were attributed to tag loss from severe abrasion, burial in sediment beyond the detection limit, or transport beyond the regions searched.

Limitations of PIT tags in field applications

Debris on the substrate and signal interference caused by nearby iron objects (Hill et al. 2006) can affect reliability of the PIT pack. The antenna configuration we used also is limited to sites with water depth <2 m. Maximum effective depth and antenna range are not necessarily uniform among sites; these limitations should be identified at each field site so that mussel absence can be distinguished from nondetection caused by equipment limitations. Reducing the antenna size for use while snorkeling, waterproofing the PIT pack for diver use, and lengthening the antenna handle are modifications that will broaden field use of this tool. At present, PIT tag use is limited to larger mussels (>20 mm length). However, smaller tags with greater detection ranges are in development, and eventually it should be possible to tag smaller mussels, at least

externally. Although internal tags were retained, the ~3-wk captive period to ensure tag retention could limit the usefulness of internal tags. Internally tagged mussels should be held in field enclosures during the initial post-tagging period when tag rejection may occur. Retaining a subset of internally tagged mussels may be a viable alternative for estimating tag retention proportions when large numbers of mussels are translocated.

The initial cost of the PIT tags and reader may exceed start-up costs for other mussel tagging methods. The PIT pack (transceivers, batteries, antenna) we used cost ~$10,000 to construct and was designed for research on a variety of organisms such as fish, mussels, and amphibians. Smaller units can be developed for ~$2500. The PIT tags we used cost $3.50 each, but the tags work indefinitely. On the other hand, the percentage of tagged mussels recaptured using PIT tags far exceeded the percentage recaptured during visual searches. Visual searches can be time-consuming and labor-intensive. For long-term monitoring of individuals and populations, the added initial costs may be recouped over time, and it may be possible to share the costs with other investigators using PIT tags.

Conclusions

PIT tags permit repeated, nondestructive sampling of individuals with little disturbance, last indefinitely, and appear to have negligible effects on short-term survival of freshwater mussels. PIT tags were retained using both internal and external attachment methods. Thus, the choice of tagging method will depend on shell thickness, habitat characteristics, and ease of implementation in the field.

The need for freshwater mussel translocations to protect and conserve threatened and endangered mussel species will increase as aquatic habitat alteration continues.

Superior recapture rates with PIT tags suggest that this tool is valuable for use in mussel translocations and monitoring and may improve accuracy of survival estimates for assessing translocation success. Because PIT tags have indefinite longevity, they can be used in monitoring both translocated mussels and populations at sites of concern, especially populations of endangered or threatened species. Moreover, because PIT tags provide reliable individual identification, they may be a useful tool for monitoring the growth and survival of individual mussels.

Chapter 3

TRANSLOCATION TO CONSERVE FRESHWATER MUSSELS FOLLOWING HABITAT ALTERATION (DAM REMOVAL) IN THE SEBASTICOOK RIVER

Introduction

North America has the greatest freshwater mussel biodiversity in the world, with nearly 300 species in the continental United States (Turgeon et al. 1988). More than 70% of these species are now considered in danger primarily due to water quality issues and habitat modifications (Williams et al. 1992). Mussels of the order Unionoida are unique in that they are obligate parasites of fish hosts during their larval (glochidial) stage. Glochidia encyst in the tissues of the fish host soon after attaching, metamorphose into juvenile mussels while attached to the fish host, then drop off to the stream bottom wherever the host is located at that stage of development. Thus, any impediment of fish dispersal, such as dams, affects freshwater mussel dispersal and distributions of mussel populations in rivers and streams.

Dams and dam removal

Dams alter the physical, chemical, and biological environments of streams, both upstream and downstream, to the point that 30-60% of the mussel fauna is destroyed or the species composition altered, primarily through the disruption of the reproductive cycle by eliminating fish host species (Williams et al. 1992). Additionally, dams trap sediments and debris that would be carried downstream by unimpeded flow. These sediments may contain large quantities of contaminants or nutrients (Stanley and Doyle

2003). An increase in nutrients may lead to eutrophic conditions in the impoundment, which few species of mussels can tolerate, and impairs visibility to the extent that mussels that use mantle lures or other host attracting strategies have difficulty attracting fish hosts (Haag and Warren 1999). The increase in sediments may result in unsuitable substrate for mussels and anaerobic conditions during low discharge periods (Blalock and Sickel 1996). Freshwater mussel species richness decreases in impoundments, with an increase in species that are more tolerant of siltation and that use a variety of common fish hosts (Miller et al. 1992, Blalock and Sickel 1996).

The Association of State Dam Safety Officers estimates that by 2020, 80% of the > 76,000 dams in the United States that are > 2 m high will require repair, replacement, or removal (Shuman 1995). Dam removal often is less expensive than repair or replacement, especially for marginally productive hydroelectric dams (Shuman 1995). Fish passage installation to enable anadromous fish migration often is a requirement for dam relicensing, with the expense exceeding projected revenues from hydroelectric power generation (Stanley and Doyle 2003).

Effects of dam removal

Shifting sediment in the impoundment is a consequence of dam removal, resulting in channel migration as rapid as dozens of meters per day (Stanley and Doyle 2003). An unstable channel may affect mussels relocated to the formerly impounded area by potentially stranding mussels in abandoned channels (Box and Mossa 1999) or burying them in sediment (Stanley and Doyle 2003).

Large-scale dam removal is a relatively recent occurrence, and studies that have examined effects of dam removal on mussels are also rare. In Wisconsin, nearly

complete mortality (95%) of mussels resulted due to stranding, desiccation, and predation when a small (3.3m high) concrete dam was removed and the impoundment dewatered (Sethi et al. 2004). Mussels below the dam also declined, due to sedimentation from the reservoir, with effects delayed as much as three years post-removal (Sethi et al. 2004). Translocation of mussels from areas where habitat alteration is expected, such as following dam removal, can be an important conservation tool. Additionally, translocated mussels must be placed in appropriate habitat, to reduce mussel loss due to voluntary mussel migration or involuntary movement due to substrate scouring (Dunn 1993, Dunn and Sietman 1993, Layzer and Gordon 1993, Cope and Waller 1995, Hamilton et al. 1997, Dunn et al. 2000).

The Fort Halifax dam and the Sebasticook River

The Sebasticook River is the largest tributary of the Kennebec River, entering the mainstem approximately 26 km upstream of Merrymeeting Bay. The mainstem of the Sebasticook is 45 km long and impounded by many hydroelectric dams. The Fort Halifax dam is located in Winslow, Maine, and is situated 427 m upstream of the confluence of the Sebasticook and the Kennebec Rivers. Constructed in 1907-1908 the dam impoundment (1.4 km^2) extends approximately 8.4 km upstream to the Benton Falls dam. FPL Energy Maine Hydro LLC (FPL Energy), the dam's owner, is seeking to partially remove the dam. In a 1987 agreement with the state, hydroelectric companies were required to install fish passages at several dams, including Fort Halifax (Richter 2003). The fish lift installation at the Fort Halifax dam was estimated to cost $4 million. Given this expense, FPL Energy opted to partially remove the dam in lieu of fish passage installation (Richter 2003). Dewatering as a result of the Fort Halifax dam removal may

result in extensive mortality of two state-listed threatened species of mussels, the yellow lampmussel (*Lampsilis cariosa*) and tidewater mucket (*Leptodea ochracea*).

Status of yellow lampmussels and tidewater muckets

The yellow lampmussel has been considered for federal listing, because it is believed to be declining throughout its range. It is also a species of special concern in Canada (Davis et al. 2004) and is listed as endangered (EN A1c) by the World Conservation Union (IUCN 1994) due to reduction in population size of at least 90% and a decline in area of occupancy, extent of occurrence and quality of habitat (Bogan 1996a). The tidewater mucket is listed as a species of special concern nationally and also is declining throughout its range. It is considered Near Threatened (NT) by the World Conservation Union (IUCN 1994, Bogan 1996b).

Both yellow lampmussels and tidewater muckets are Atlantic Slope species found historically from Georgia to New Brunswick (Nedeau et al. 2000). In Maine yellow lampmussels are found in relatively few sites (Sebasticook, St. George, middle Penobscot, and Passadumkeag River systems), and populations at these sites are reproducing and considered healthy (Nedeau et al. 2000). The largest populations of tidewater muckets in Maine are in the lower Kennebec and Penobscot River drainages (Nedeau et al. 2000). Both yellow lampmussels and tidewater muckets use a variety of substrates, including silt, sand, gravel, and cobble. Yellow lampmussels are found in medium to large rivers, and also occur in ponds, streams and impoundments (Nedeau et al. 2000). Tidewater muckets are found primarily in coastal lakes, ponds, and slow moving rivers, including impoundments (Nedeau et al. 2000). Populations of these two species in Maine are reproducing and are relatively undisturbed compared to elsewhere in

their range, so Maine populations may represent a stronghold for these species (Nedeau et al. 2000).

Mussel translocations

A goal in the National Strategy for the Conservation of Native Freshwater Mussels is to "develop, evaluate, and use the techniques necessary to hold and translocate large numbers of adult mussels" (National Native Mussel Conservation Committee 1997). Current knowledge of translocation effectiveness is limited (Dunn 1993, Layzer and Gordon 1993, Cope and Waller 1995, Dunn and Sietman 1997, Hamilton et al. 1997, Dunn et al. 2000). Of particular importance is the difficulty in recapturing translocated mussels (although, see Kurth et al. 2007, Chapter 2), which may result in artificially low population and survival estimates.

Previous mussel translocations in North America have been due primarily to bridge construction or repair, and the mussels were simply translocated upstream of the area affected by the construction (Havlik 1997, Bolden and Brown 2002, Cope et al. 2003). This approach might not always be possible in the case of dam removals, given that a free-flowing environment often will replace a large impounded area. In question is whether translocations should be limited to within-waterbody when restoration of the waterbody is the intended result. Mussel survival in within-waterbody translocations versus translocation between waterbodies has not been adequately studied.

Survival estimates of translocated mussels often are based on the number of mussels recaptured or found dead, with non-recaptured mussels assumed to have emigrated from the study site (Dunn 1993, Layzer and Gordon 1993, Cope and Waller 1995, Dunn and Sietman 1995, Hamilton et al. 1997, Dunn et al. 2000). Low recapture

rates in translocation studies may result in inflated estimated survival rates (Cope and Waller 1995). Passive Integrated Transponder (PIT) tags are an effective tool for recapturing translocated mussels and increasing accuracy of survival estimates (Kurth et al. 2007, Chapter 2).

Proposed Incidental Take Plan

FPL Energy was required to submit an Incidental Take Plan (ITP) proposing methods to limit the mortality of yellow lampmussels and tidewater muckets due to the Fort Halifax dam removal, because the impoundment contains these state-listed species. As required in the ITP, FPL Energy plans to move all yellow lampmussels and tidewater muckets found on the dewatered, exposed substrate to sites in Sandy Stream and Unity Pond (Fig. 3.1). Sandy Stream is a first-order, spring-fed stream that drains into Unity Pond. Unity Pond is a 1039-ha lake that joins the Sebasticook River ~30 km upstream of the Fort Halifax dam. Yellow lampmussels and tidewater muckets are found in both waterbodies, and populations of both species are genetically similar at neutral markers among sites (Kelly 2004). The ITP requires that FPL Energy monitor 60 yellow lampmussels and 60 tidewater muckets translocated to Sandy Stream, as well as the same number native to Sandy Stream. The remaining recovered mussels will be moved to Unity Pond with no monitoring. Mussel survival in within-waterbody translocations versus translocation between waterbodies rarely has been studied. The objectives of this study are to evaluate survival of mussels translocated within-waterbody versus between waterbody (from the Sebasticook River to Unity Pond and to Sandy Stream), and to develop guidelines for mussel translocations during the Fort Halifax dam removal.

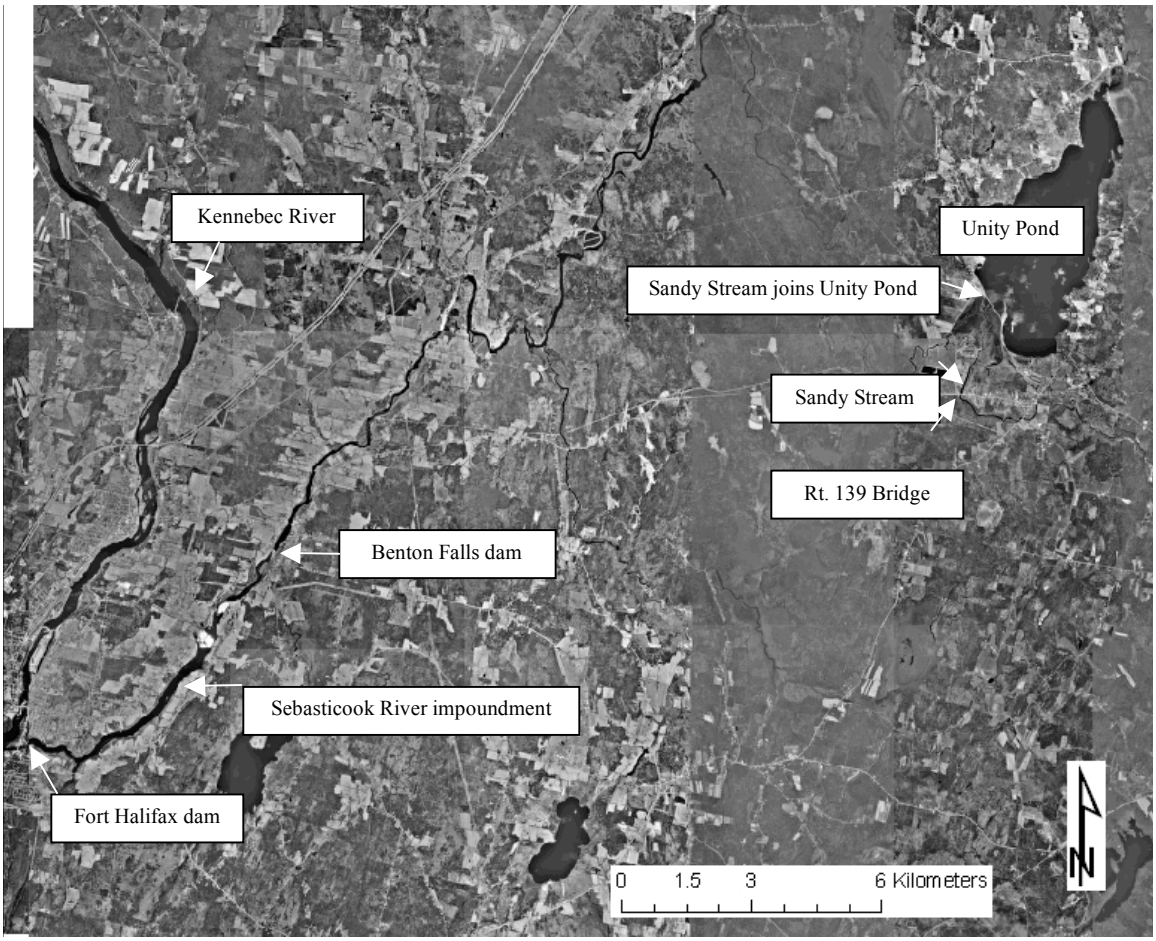


Figure 3.1. Location of waterbodies for translocation studies. See Figure 3.2 for details of translocation sites.

Methods and Materials

Eastern lampmussel translocations

I conducted a pilot translocation study with eastern lampmussels (*Lampsilis radiata radiata*) during September-October 2004 to examine effects of moving mussels within and between waterbodies on mussel survival. I conducted three types of translocations at sites identified in the ITP within the Fort Halifax dam impoundment of the Sebasticook, Sandy Stream, and Unity Pond: within waterbody, between waterbodies, and no movement (control) (Fig. 3.1).

I collected 121 eastern lampmussels from the Sebasticook River impoundment (Fig. 3.2a), measured mussel length, width, and thickness (to nearest mm), and tagged each mussel with a PIT tag and numbered bee tag (Kurth et al. 2007, Chapter 2). Due to the abundance of eastern lampmussels in the impoundment, I collected larger individuals (> 50 mm). I replaced 30 mussels where they were found in the impoundment and 30 mussels in three 1m x 2m plots located ~2.5 km below the Benton Falls dam (Fig. 3.3a). I marked the plots with stakes and flagging at the corners, and I used a Global Positioning System (GPS) to record the plot corner locations and the locations of each picked up and replaced mussel. The plot substrate was fine silt and patches of vegetation.

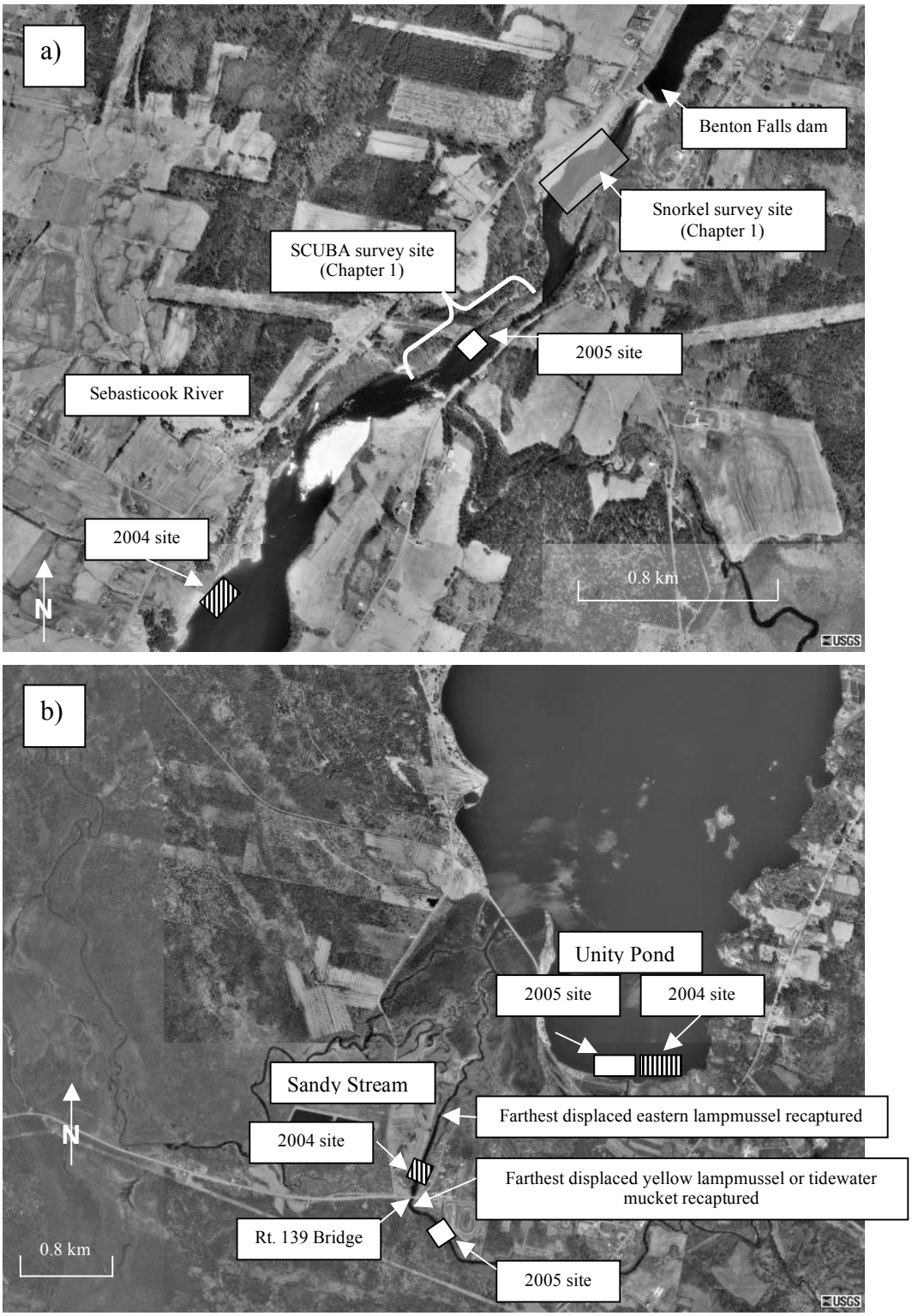
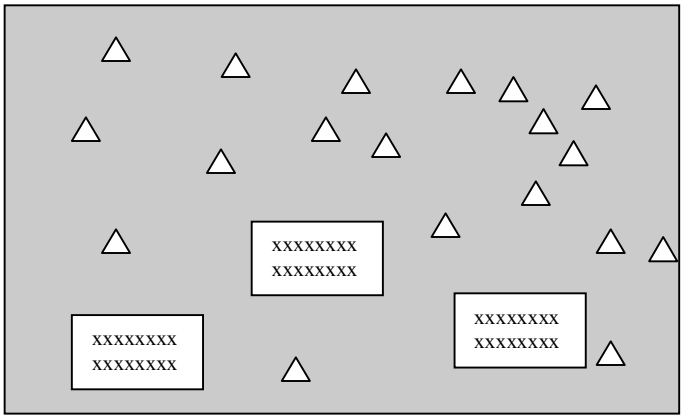
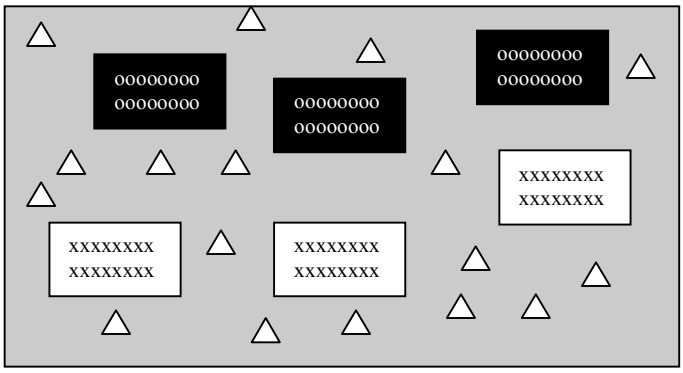


Figure 3.2. Translocation study sites at (a) Sebasticook River and (b) Sandy Stream and Unity Pond for eastern lampmussels in 2004 and yellow lampmussels and tidewater muckets in 2005.



Sebasticook River:
△ = picked up and replaced mussels

x = mussels moved within the Sebasticook

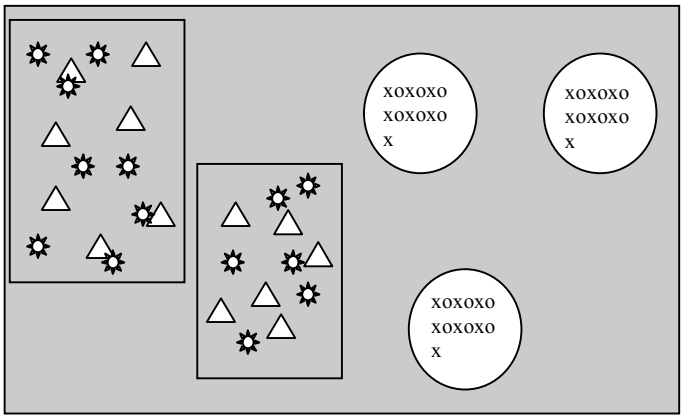


Unity Pond and Sandy Stream:
△= picked up and replaced mussels

x = mussels moved within Unity Pond or Sandy Stream

o = mussels moved from the Sebasticook River

(a)



All sites (2005):
△= picked up and replaced yellow lampmussels in flag delineated area

✡= picked up and replaced tidewater muckets in flag delineated area

x = yellow lampmussels moved from the Sebasticook River (or within the impoundment) and placed in flag-centered plot

o = tidewater muckets moved from the Sebasticook River (or within the impoundment) and placed in flag-centered plot.

(b)

Figure 3.3. Schematic diagram of translocation study design for (a) eastern lampmussel translocations in the Sebasticook River and Unity Pond and Sandy Stream (2004), and (b) yellow lampmussels and tidewater muckets in the Sebasticook River, Sandy Stream, and Unity Pond (2005).

I moved 29 of the tagged mussels from the Sebasticook River to the southern end of Unity Pond (Fig. 3.2b) and randomly distributed them among three 1 m x 2 m plots (Fig. 3.3a). I collected 61 eastern lampmussels (length > 50 mm) in Unity Pond, tagged them with PIT tags and bee tags, and randomly distributed 31 among the plots containing mussels translocated from the Sebasticook River. The remaining 30 mussels were replaced in their collection location. I marked the plot corners with stakes and flagging, and I recorded locations of each plot and for each of the picked up and replaced mussels with GPS. The plot substrate was sandy with patches of vegetation.

I moved 32 tagged mussels from the Sebasticook River to Sandy Stream immediately downstream of the Rt. 139 Bridge (Fig. 3.2b) and randomly distributed them among three 1 m x 2 m plots (Fig. 3.3a). Eastern lampmussels in Sandy Stream were less common, or a greater proportion may have been burrowed into the substrate due to colder water temperatures. The eastern lampmussels I collected at this site were smaller (> 40 mm) than those collected at the other two sites. I collected 56 eastern lampmussels in Sandy Stream, tagged them with PIT tags and bee tags, and randomly distributed 26 among the plots. I replaced the remaining 30 mussels at their collection location. I marked the plot corners with stakes and flagging, and I recorded locations for each plot and for each picked up and replaced mussel (control) with GPS. I also marked the sites of the control mussels with stakes and flagging. The plot substrate was sand and gravel with patches of silt.

During June-August 2005 I recaptured mussels to determine survival one year post-translocation (Kurth et al. 2007, Chapter 2). I searched for mussels at the Unity Pond and Sebasticook River study sites for ~25 hours over 4 days to at least 3 m beyond the study area perimeter at each site. I also searched shorelines for valves from dead mussels. Extensive ice scouring and spring flooding substantially reconfigured the Sandy Stream substrate, so I also searched for ~18 hours over 3 days from bank to bank to 200 m downstream of the site to locate mussels displaced by high flows. I repeated the searches during July-August 2006, searching 18-20 hours over 3 days at each site.

Yellow lampmussel and tidewater mucket translocations

I repeated the translocation study with yellow lampmussels and tidewater muckets during July-August 2005. I modified the methods by eliminating the within-waterbody translocation group at Unity Pond and Sandy Stream, improving study area markings, and establishing new study plots within the waterbodies where I found concentrations of yellow lampmussels and tidewater muckets. In contrast with eastern lampmussels, yellow lampmussels and tidewater muckets were less common at the study sites. I tagged each mussel with a PIT tag and numbered bee tag (Kurth et al. 2007, Chapter 2) and measured length, width, and thickness of all individuals of these species that I found, regardless of size, so that sample sizes would be sufficient for the tagging and translocation experiment.

I established three 4 m diameter study plots each centered on a flagged stake in the Sebasticook River ~ 1 km below the Benton Falls dam in cobble and boulder substrate with patchy submerged vegetation (Fig. 3.2a). I collected 126 yellow lampmussels and 134 tidewater muckets from the impoundment, and randomly placed 31

yellow lampmussels and 37 tidewater muckets in the plots (Fig. 3.3b). I replaced 28 yellow lampmussels and 33 tidewater muckets where I found them in the impoundment (Fig. 3.3b). I recorded locations of the plots and corners of the area containing the replaced mussels with GPS, and I marked the replacement areas with flagged stakes. The remaining tidewater muckets and yellow lampmussels were translocated to Unity Pond and Sandy Stream.

I randomly placed tagged yellow lampmussels (34) and tidewater muckets (31) collected from the Sebasticook River into three 4 m diameter plots (centered on a flagged stake) in sandy substrate and patchy vegetation in Unity Pond (Fig. 3.2b and 3.3b) and tagged and replaced additional yellow lampmussels (30) and tidewater muckets (31) in Unity Pond where they were found. Yellow lampmussels and tidewater muckets were not evenly distributed in Unity Pond; two areas selected to collect marked and replaced mussels contained more tidewater muckets, whereas the third area contained only yellow lampmussels. I defined the corners of the areas containing these mussels with flagged stakes, and I recorded their locations with GPS (Fig. 3.3b).

I relocated the Sandy Stream study site ~275 m upstream from the site used in the pilot study (Fig. 3.2b) to avoid loss of tagged mussels that occurred when the substrate downstream from the bridge was redistributed during high flows in winter 2004-spring 2005. The relocated study area had sandy and rocky ledge substrate with ~0.75 m water depth, and rocky ledge and patches of sand/gravel with ~1-2 m water depth. I randomly placed tagged yellow lampmussels (33) and tidewater muckets (33) collected from the Sebasticook River into sandy substrate in three 4 m diameter plots centered on flagged stakes (Fig. 3.3b). I collected, tagged, measured, and replaced an additional 37 yellow

lampmussels and 28 tidewater muckets that I found distributed in the region of the plots (Fig. 3.3b). Tidewater muckets and yellow lampmussels were not evenly distributed in this section of Sandy Stream; most (26) tidewater muckets were found in an area with sandy substrate, whereas yellow lampmussels were found in sandy areas and ledge habitat with sandy pockets. I divided the area containing the tagged and replaced mussels into 3 sections to facilitate relocations. I marked the section corners with flagged stakes, and I recorded the coordinates of the stakes with GPS.

I recaptured mussels during July-August 2006 to determine effects of translocation on mussel survival. I searched for tagged mussels at the Sebasticook River and Unity Pond sites for 18-20 hours over 3 days at each site, including the area 3 m beyond the study site perimeter. I searched for tagged mussels at the Sandy Stream site for 18-20 hours over 3 days to ~125 m downstream of the 2004-2005 eastern lampmussel pilot study area (Figure 3.2b). I also searched the shoreline and muskrat (*Ondatra zibethicus*) middens for tagged valves at each study area. I also noted numbers of mussels found visible on the substrate surface and those found completely burrowed into the substrate.

Data Analysis

I compared mussel mortality among treatments and controls for both yellow lampmussels and tidewater muckets with an adjusted χ^2 for small sample sizes (Gotelli and Ellison 2004). I also calculated an analysis of variance ($\alpha = 0.05$) with a post-hoc Bonferroni adjustment to determine if there were differences among sites in sizes of yellow lampmussels and tidewater muckets. Differences were deemed significant if $p < 0.05$.

Results

Eastern lampmussel translocations

During June-July 2005 and July-August 2006 I recaptured 83% of the tagged eastern lampmussels at the Sebasticook River including two dead mussels (Table 3.1). One of the dead tagged mussels was found on the shore of the Sebasticook River. I recaptured 77% of the tagged eastern lampmussels at Unity Pond, and I found no dead mussels at this site. Only 34% of tagged eastern lampmussels were recovered at Sandy Stream, and 27% of the recaptured mussels had died. Ice scouring and spring flooding reconfigured the substrate at this site, and I found 71% of recaptured mussels >100 m from their October 2004 locations. I recaptured mussels at Unity Pond and the Sebasticook River ≤ 2 m from their September-October 2004 locations.

The size distribution for eastern lampmussels as a percentage of the mussels collected from each site is presented in Figure 3.4. While collecting eastern lampmussels at Unity Pond and the Sebasticook River, I searched for mussels ≥ 50 mm length due to their abundance; therefore, mussels ≤50 mm are excluded from the population size distribution (Fig. 3.4). Most eastern lampmussels I found on Sandy Stream substrates were > 50 mm length (Fig. 3.4).

Table 3.1. Number of recaptured and dead translocated eastern lampmussels by year and treatment in the Sebasticook River watershed, Maine, 2005 and 2006.

			2005		2006		overall	
site	**treatment**	**# tagged**	**# captured**	**mortality**	**# captured**	**mortality**	**# captured**	**mortality**
Sebasticook	Moved within	30	24	0	26	0	30	0
	Picked up/replaced	30	18	1	13	1	20	2
Unity Pond	Moved within	31	21	0	17	0	23	0
	Picked up/replaced	30	15	0	12	0	19	0
	From Sebasticook	29	23	0	22	0	27	0
Sandy Stream	Moved within	26	4	0	5	2	9	2
	Picked up/replaced	30	8	0	5	2	9	2
	From Sebasticook	32	9	1	5	3	12	4

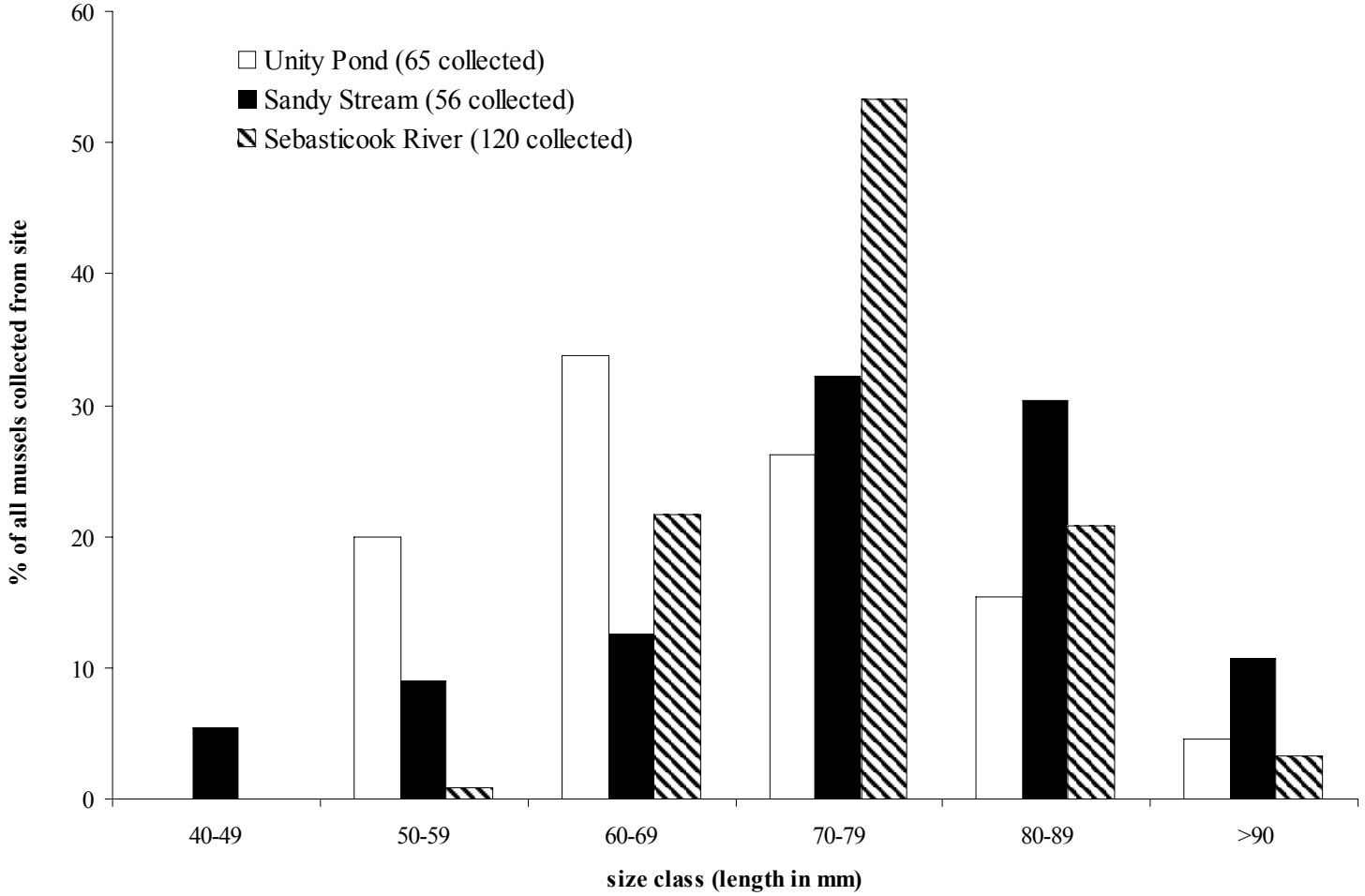


Figure 3.4. Size distribution of eastern lampmussels when collected from each waterbody, as a percentage of all mussels collected from that waterbody during August-September 2004.

Yellow lampmussel and tidewater mucket translocations

During July-August 2006 I recaptured 90% of yellow lampmussels (7% mortality) and 71% of tidewater muckets (4% mortality) in the Sebasticook River and 88% of yellow lampmussels (no mortality) and 85% of tidewater muckets (6% mortality) at Unity Pond (Table 3.2). I recaptured only 57% of yellow lampmussels (1% mortality) and 30% of tidewater muckets (5% mortality) at Sandy Stream, where the substrate was modified substantially between mussel release and recapture. Mortality of tidewater muckets moved to Sandy Stream exceeded that of tidewater muckets retained in the Sebasticook River (adj. $\chi^2 = 9.5$, $p = 0.0496$, d.f. = 4) but did not meet the criteria for significance when compared to tidewater muckets native to Sandy Stream (adj. $\chi^2 = 8.33$, $p = 0.0803$, d.f. = 4). Mortality was not significantly different among other treatment groups.

During August 2006, I found 2 yellow lampmussels and 4 tidewater muckets 30-100 m downstream from their August 2005 locations in Sandy Stream, whereas recovered mussels at Unity Pond and the Sebasticook River moved < 4 m from their August 2005 locations. I found no tagged mussels in middens at Sandy Stream, despite evidence of muskrat predation on yellow lampmussels and tidewater muckets at the site. Mussel valves recovered at Sandy Stream either were buried in the substrate or contained fresh tissue, suggesting that these deaths did not result from predation. I found one dead tagged yellow lampmussel in a muskrat midden on the Sebasticook River shoreline.

Table 3.2. Number of recaptured or dead yellow lampmussels and tidewater muckets translocated during 2005-2006 to water bodies in the Sebasticook River watershed, Maine.

		yellow lampmussels			tidewater muckets		
site	**treatment**	**# tagged**	**# captured**	**mortality**	**# tagged**	**# captured**	**mortality**
Sebasticook River	Moved within	31	29	1	33	24	2
	Picked up/replaced	28	24	3	37	26	1
Unity Pond	From Sebasticook	34	30	0	31	29	3
	Picked up/replaced	30	26	0	31	24	1
Sandy Stream	From Sebasticook	33	14	1	33	8	1
	Picked up/replaced	37	26	1	28	11	2

At Unity Pond in July, I found 29% of tidewater muckets and 23% of yellow lampmussels completely burrowed into the substrate, and by August all mussels were found at the substrate surface. In the Sebasticook River in August, I found only 8% of tidewater muckets and 1% of yellow lampmussels burrowed in the substrate. In Sandy Stream, which is colder than the Sebasticook River and Unity Pond regardless of month, 53% of tidewater muckets and 80% of yellow lampmussels were completely burrowed in the substrate.

Yellow lampmussels collected from Sandy Stream (58 $\pm$ 14 mm) were significantly smaller ($F = 24.404$, $p < 0.0001$ with a post hoc Bonferroni adjustment) than those from Unity Pond (73 $\pm$ 15 mm) and the Sebasticook River (75 $\pm$ 12 mm) (Fig. 3.5). Additionally, tidewater muckets collected from the Sebasticook River (60 $\pm$ 10 mm) were significantly larger ($F = 21.891$, $p < 0.0001$ with a post hoc Bonferroni adjustment) than those from Unity Pond (58 mm $\pm$ 14 mm) and Sandy Stream (49 $\pm$ 12 mm). Individuals < 25 mm were not found on the substrate surface and presumably were burrowed (Balfour and Smock 1995, Amyot and Downing 1997).

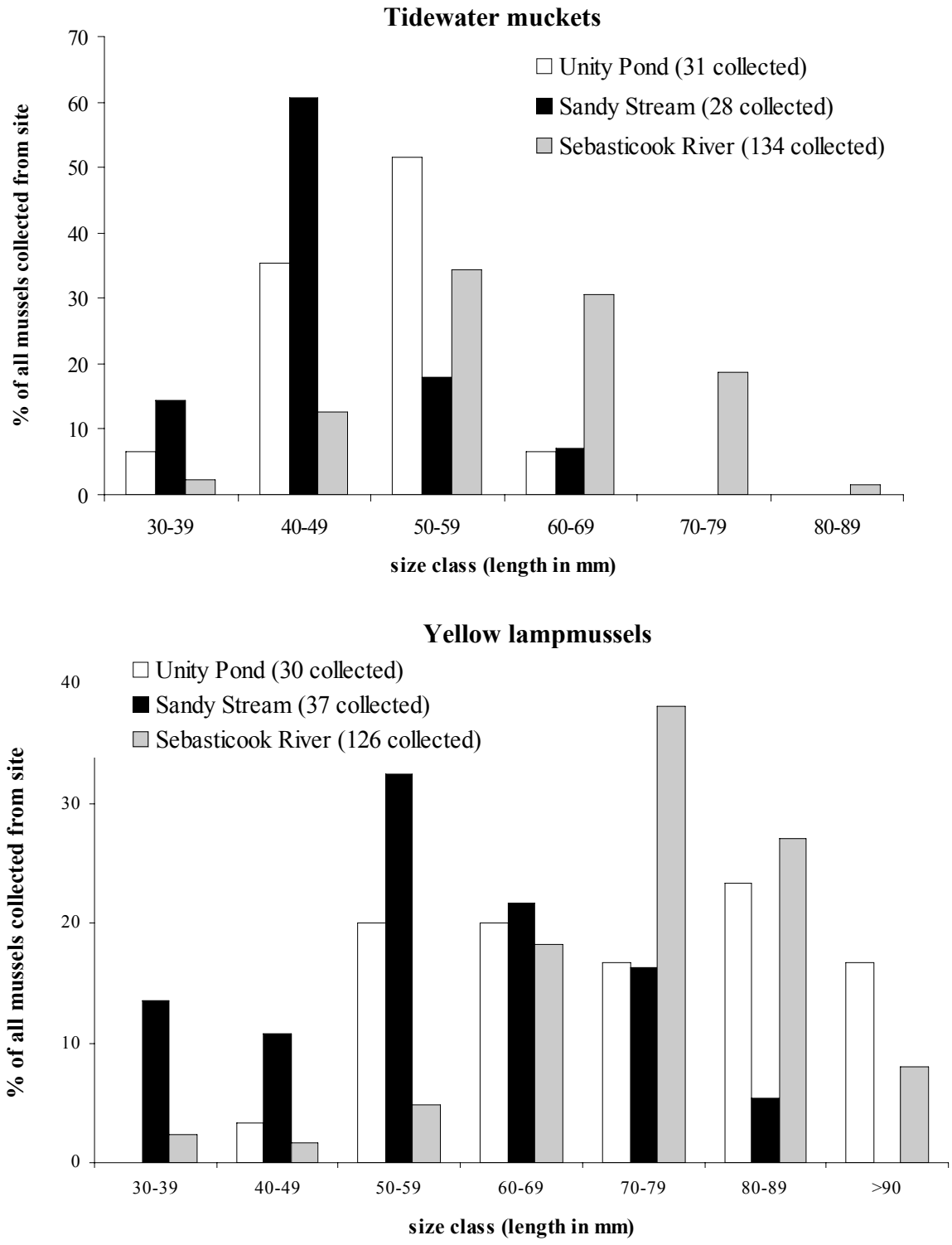


Figure 3.5. Size distribution of yellow lampmussels and tidewater muckets when collected from each waterbody, as a percentage of all mussels collected from that waterbody during August 2005.

Discussion

Evaluation of eastern lampmussel, yellow lampmussel, and tidewater mucket translocations

More eastern lampmussels, yellow lampmussels, and tidewater muckets were recaptured in the Sebasticook River and Unity Pond than in Sandy Stream. Sandy Stream was identified in the ITP as a translocation site for yellow lampmussels and tidewater muckets that will be exposed during impoundment dewatering following removal of the Fort Halifax dam (Richter 2003). In this study, only a third (30 of 88) of tagged eastern lampmussels were recaptured 24 months after translocation to Sandy Stream, and recaptures of tagged yellow lampmussels (40 of 70) and tidewater muckets (19 of 63) also were low 12 months after translocation to Sandy Stream. The fate of non-recaptured mussels is not known; Sandy Stream sediments were redistributed by winter and spring high flows, and it is likely that the mussels were transported with the moving sediment. Tagged mussels may have survived displacement beyond the searched area, or they may have been buried deeper than the detection limit of the PIT tag receiver (~0.5 m) by shifting sediments. The instability of the Sandy Stream substrates potentially threatens mussel survival, and I do not recommend using the site as a destination for translocations.

Wick (2006) quantitatively sampled yellow lampmussels and tidewater muckets at Sandy Stream and found them in low densities in contrast to population densities in Unity Pond and the Sebasticook River impoundment. In my study, numbers of recaptured, tagged tidewater muckets, yellow lampmussels, and eastern lampmussels also were greater in the Sebasticook River impoundment and Unity Pond than in Sandy

Stream. Low survival of mussels during the annual reconfiguration of Sandy Stream substrates may contribute to low population numbers, as indicated by low recaptures.

Post-translocation mussel mortality has been reported in the year following translocation (Dunn 1993), as well as two to three years post-translocation (Layzer and Gordon 1993, Newton et al. 2001). Long term monitoring is imperative to identify delayed responses of mussels to translocations (Dunn 1993, Layzer and Gordon, 1993, Cope and Waller 1995, Newton et al. 2001). PIT tag longevity is indefinite, so mussels tagged in this study could be monitored to evaluate long-term survival. Additionally, PIT tags allow for relatively easy detection of burrowed mussels. I found variation in the numbers of burrowed yellow lampmussels and tidewater muckets that appears to be related to water temperature. The use of PIT tags enhances mussel recapture regardless of the effects of water temperature on burrowing of mussels.

Yellow lampmussels and tidewater muckets were monitored only one year post-translocation, whereas eastern lampmussels were monitored for two years. Numbers of mussels recaptured were relatively consistent between years (Table 3.1), particularly for mussels placed in plots at Unity Pond and the Sebasticook River. Mussel recaptures in Sandy Stream were slightly greater the first year, and it is likely that tagged mussels were redistributed by moving sediments in Sandy Stream between the two recapture periods. I recaptured 6 eastern lampmussels during the second survey year that I did not find in searches after tagging during the first survey year, although 5 of them were dead. I also found eastern lampmussels during the second survey year that I did not recapture following release of tagged mussels in the first survey year at Unity Pond (n=8) and the Sebasticook River (n=6), where the substrates were not significantly reconfigured,

emphasizing the need to repeatedly survey any translocation site to accurately assess survival.

Differences in growth and condition of translocated mussels may indicate differences in site quality. Growth may provide an appropriate assessment of habitat quality for small mussels, which have greater potential to grow more, proportionally, than large mussels (Newton et al. 2001). Shell growth rates may be controlled genetically, whereas shell shape can be modified by environmental factors (Hinch et al. 1986). Changes in certain physiological measures, such as glycogen levels, often can precede changes in mussel survival (Monroe and Newton 2001). Tissue glycogen concentrations may quantify sub-lethal effects of translocation on mussels; increased energy demands associated with translocation stress may result in depleted glycogen reserves (Patterson et al. 1997, Naimo et al. 1998, Patterson et al. 1999) (see Appendix B). Glycogen is the primary source of energy for mussels and can be used to survive short-term emersion, anoxia, or starvation (Patterson et al. 1999). Glycogen concentration has been used as a bioindicator of condition in studies examining mussel emersion stress (Chen et al. 2001), quarantine and translocation effects (Patterson et al. 1997, Naimo et al. 1998, Patterson et al. 1999), effects of zebra mussel infestation (Hallac and Marsden 2000), effects of artificial diets on captive mussels (Nichols and Garling 2002), and seasonal variation in mussel condition (Monroe and Newton 2001). Glycogen levels may vary with gender, especially during breeding and brooding by females (Hallac and Marsden 2000, Monroe and Newton 2001).

Size distributions of mussels

Because I was searching for mussels on the substrate surface, I found no yellow lampmussels or tidewater muckets < 25 mm, a size below which most mussels remain burrowed (Amyot and Downing 1991, Balfour and Smock 1995). Although populations of these species appear to be reproducing at these three sites, yellow lampmussels and tidewater muckets collected from Sandy Stream were smaller (yellow lampmussels: 56% < 59mm; tidewater muckets: 93% < 59mm) than yellow lampmussels (9% <59mm) and tidewater muckets (49% < 59mm) collected at the Sebasticook River and yellow lampmussels (23% <59mm) collected at Unity Pond. Tidewater muckets collected at Unity Pond (94% < 59mm) and those found at Sandy Stream were similar in size. Wick (2006) also found that yellow lampmussels and tidewater muckets were smaller in Sandy Stream than in the Sebasticook River impoundment, although the differences in tidewater mucket sizes were not as extreme as those I saw. Tidewater muckets and yellow lampmussels may grow more slowly at Sandy Stream than at other sites, or the population structure could be skewed towards smaller size classes because of high mortality of larger individuals, likely due to muskrat predation.

Mussel feeding is a complicated, dynamic process that may differ by environment, species, and life stage and has consequences for mussel conservation; mussel feeding habits and quantitative assessments of food quality and quantity across habitats remains unstudied (Strayer et al. 2004). There may be more food, or higher quality food, for mussels in the Sebasticook River and Unity Pond than in Sandy Stream. Sandy Stream is a cold, spring-fed stream with clear water and no obvious algal blooms, whereas the Sebasticook River is warm and subject to occasional algal blooms during the

summer, and Unity Pond is warm and eutrophic for most of the summer (personal observation). Few species of mussels can tolerate eutrophic conditions, and impaired visibility may hamper reproducing mussels, such as the yellow lampmussel, which use mantle lures or other host attracting strategies (Haag and Warren 1999).

Strategies to improve mussel recapture

Using PIT tags increased numbers of recaptured mussels (Kurth et al. 2007, Chapter 2), and adding a visual marker to define the area or plot center also improved recapture efficiency by providing a reference from which searches for tagged mussels could begin, since GPS accuracy was inconsistent (1-3 m). A greater proportion of tagged (translocated) mussels placed in plots were recaptured than mussels that were picked up and replaced (control), because the control mussels were more widely distributed at the study sites. Cope et al. (2003) found that doubling or tripling the density of mussels did not adversely affect survival, suggesting that increasing the density of tagged mussels to facilitate recapture success would not be detrimental, although this depends on the quality of the translocation site. It may be possible to place control mussels in plots within the area from which they were collected without adversely affecting survival, thus improving recaptures of these mussels.

Recommendations for post-dam removal translocations

Relocated mussels, especially older individuals, may not acclimate if the habitat of the destination site differs from the source habitat (Cope and Waller 1995). Ideally, habitat at the translocation site should be similar to that from which the mussels are removed (Cope and Waller 1995). The presence of a healthy, reproducing population of the target species at the translocation site indicates that appropriate fish hosts are present,

which is critical to a successful translocation (Villella et al. 1998). However, not all sites meeting these qualities are of equal value for conservation. Translocated mussels ideally should be placed in habitat where mussel loss due to substrate scouring or predation is minimized (Dunn 1993, Layzer and Gordon 1993, Cope and Waller 1995, Dunn and Sietman 1995, Hamilton et al. 1997, Dunn et al. 2000). Sandy Stream is spring-fed and subject to high flows during late winter-early spring. Stream sediment is redistributed annually with this high flow, which carries debris and stream-dwelling organisms downstream toward Unity Pond. Although tidewater muckets and yellow lampmussels occur in this stream, the unstable stream bottom and possible muskrat predation potentially threatens their survival, making this site less desirable for translocating yellow lampmussels and tidewater muckets from the Sebasticook River in preparation for removal of the Fort Halifax dam. Additionally, the ITP requires that translocated mussels be monitored; although PIT tags increase relocation efficiency (Kurth et al. 2007, Chapter 2), mussels that have been transported great distances downstream are not likely to be recaptured. Unity Pond is subject to eutrophic conditions and very warm water temperatures during the summer, which may make it less than ideal as a translocation site.

Channel instability following dam removal depends on the type and distribution of sediment fill in the impoundment (Pizzuto 2002, Stanley and Doyle 2003). The ITP proposes translocating tidewater muckets and yellow lampmussels to Unity Pond and Sandy Stream in anticipation of post-dam removal instability in the Sebasticook River channel (Richter 2003). The substrate in the upper 1.5 km of the impoundment, where I found the greatest densities of yellow lampmussels and tidewater muckets (Chapter 1),

primarily is boulders and cobble. This area is least likely to be reconfigured following dam removal, because the boulders and cobble in the channel should be stable during dewatering. Mussels in this area are probably at little risk of exposure, if moved to the channel during dam removal. Dewatering in the lower ~1.5 km of the impoundment is expected to redistribute the fine silt behind the Fort Halifax dam. Tidewater muckets and yellow lampmussels were sparsely distributed in this area (Chapter 1), so few of these mussels are expected to be affected by dewatering this section of the impoundment. Channel migration is expected in the middle 5.5 km of the impoundment, where the substrates are silt and mud. The impoundment width ranges 200-400 m in this section, with shallow (1-2 m depth) mudflats spanning more than half the impoundment width. Water depths in what was likely the original channel before dam construction currently range 4-7 m. During qualitative surveys I found low numbers of yellow lampmussels and tidewater muckets in this section of the impoundment, as well as tens of thousands of eastern lampmussels, eastern floaters (*Pyganodon cataracta*) and eastern elliptio (*Elliptio complanata*) (Chapter 1, Appendix A). In this impoundment section mussels are at risk of exposure, stranding, and burying by the meandering channel and redistributed sediments during and following drawdown, so mussels should not be moved to this area for conservation purposes.

Given the expected channel stability in the upper section of the impoundment and the unsuitability of Unity Pond and Sandy Stream, survival of mussels exposed during dewatering may be greatest if they are moved within the Sebasticook to this more stable channel. This area may be a refuge from which all mussels species found in the impoundment can then repopulate the newly formed channel once it stabilizes in the

middle of the impoundment. Alternatively, once the mid-impoundment channel stabilizes, mussels could be translocated from this refuge to the new channel to accelerate the repopulation of the channel or to decrease mussel densities in the upper impoundment. Other studies have shown, however, that doubling or tripling the density of mussels did not adversely affect mussel survival (Cope et al. 2003). Additionally, we estimate densities of 0.272-0.389/m^2 for yellow lampmussels and 0.094-0.366/m^2 for tidewater muckets in the SCUBA surveys (Chapter 1), although we found densities up to two times greater in snorkel survey areas, as did Wick (2006), suggesting that these species can tolerate higher densities.

Because PIT tags improve recapture of translocated mussels, they should be used in the translocated yellow lampmussels and tidewater muckets that are to be monitored. PIT tags permit repeated, nondestructive sampling of individuals with little disturbance, and they last indefinitely. This application is ideal for the long-term monitoring (10-20 y) needed to assess the effects of the dam removal on these mussels. In addition to growth and survival, some assessment of physiological condition, such as tissue glycogen concentration, should be conducted as this provides a finer-scale measurement of the effects on the dam removal and translocation of mussels. Finally, the translocated mussels will need to be monitored to determine if they are reproducing following translocation. If there is no reproduction, translocation will only have delayed the death of these species in the impoundment, instead of saving them. Dam removal and the dewatering and channel instability that follows can be detrimental to mussels; however, restoring free-flowing habitat will increase access to fish hosts and improve the system as a whole, which ultimately will benefit mussels (Stanley and Doyle 2003).

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APPENDIX A.

QUALITATIVE AND QUANTITATIVE SURVEYS OF MUSSELS IN THE FORT HALIFAX DAM IMPOUNDMENT, SEBASTICOOK RIVER, MAINE, 2004-2005.

Table A.1. All mussel species found in 2004 qualitative surveys (SCUBA plus snorkel and snorkel only) of the Fort Halifax dam impoundment of the Sebasticook River, Maine. Numbers of eastern lampmussels, eastern elliptio, and eastern floaters are estimates. Survey methods are detailed in Chapter 1.

Section*	Substrate	*Lampsilis cariosa* **Yellow Lampmussel**	*Leptodea ochracea* **Tidewater Mucket**	*Lampsilis radiata radiata* **Eastern Lampmussel**	*Elliptio complanata* **Eastern Elliptio**	*Pyganodon cataracta* **Eastern Floater**
Upper 1.5 km of impoundment	Boulder and cobble	97	40	100s	100s	100s
Middle section of impoundment (~5.5 km long)	Silt/mud flat	21	31	10,000s	10,000s	1000s
Lower 1.5 km of impoundment from China Lake Outlet to Fort Halifax dam	Silt/sand/steep sides	2	1	1000s	1000s	1000s

*sections indicated on Figure 1.1.

Table A.2. Population and density estimates for selected mussel species found in 2004 and 2005 quantitative snorkel surveys in the Fort Halifax dam upper impoundment in the Sebasticook River, Maine. See Chapter 1 for population and density estimates for yellow lampmussels and tidewater muckets and methods used to calculate these estimates.

Site[a]	Date		*Lampsilis radiata* Eastern Lampmussel	*Elliptio complanata* Eastern Elliptio	*Pyganodon cataracta* Eastern Floater	*Anodonta implicata* Alewife Floater	*Alasmidonta undulata* Triangle Floater
1	July 05	Population Estimate	981	341	107	149	85
		90% CI	362-2660	58-2028	60-189	66-337	42-174
		Density Estimate (/m^2)	0.785	0.273	0.085	0.119	0.068
1	Aug 05	Population Estimate	1579	277	21	917	192
		90% CI	608-4098	126-612	1-369	487-1727	45-820
		Density Estimate (/m^2)	1.263	0.222	0.017	0.734	0.154
2	July 05	Population Estimate	85	149	0	21	0
		90% CI	13-562	51-439	-	1-369	-
		Density Estimate (/m^2)	0.068	0.119	0.000	0.017	0.000
3	Aug 04	Population Estimate	2133	1216	192	0	21
		90% CI	1436-3170	769-1923	64-575	-	1-369
		Density Estimate (/m^2)	1.707	0.973	0.154	0.000	0.017
3	July 05	Population Estimate	4907	3712	64	149	235
		90% CI	4214-5714	2943-4683	64-64	99-224	43-1284
		Density Estimate (/m^2)	3.925	2.970	0.051	0.119	0.188
3	Aug 05	Population Estimate	4117	1024	0	725	64
		90% CI	3095-5477	707-1484	-	455-1157	12-332
		Density Estimate (/m^2)	3.294	0.819	0.000	0.580	0.051
4	July 05	Population Estimate	1024	192	43	21	43
		90% CI	345-3042	111-332	3-738	1-369	10-177
		Density Estimate (/m^2)	0.819	0.154	0.034	0.017	0.034
5	Aug 05	Population Estimate	725	64	0	192	21
		90% CI	581-905	12-332	-	111-332	1-369
		Density Estimate (/m^2)	0.580	0.051	0.000	0.154	0.017

[a] Site numbers refer to plot locations in Figure 1.2.

APPENDIX B

EFFECTS OF INTERNAL PIT TAGGING ON GLYCOGEN LEVELS IN FRESHWATER MUSSELS

Introduction

Traditionally, measures of mussel health have been limited to growth and survival; however, these measures may not be sufficiently sensitive to accurately assess changes in mussel condition. Changes in certain physiological measures, such as glycogen levels, often precede mussel death (Monroe and Newton 2001). Glycogen is the primary energy source for mussels and can be used to survive short-term exposure to emersion, anoxia, or starvation (Patterson et al. 1999). Glycogen concentration has been used as a bioindicator of condition in many mussel studies examining emersion stress (Chen et al. 2001), quarantine and translocation effects (Patterson et al. 1997, Naimo et al. 1998, Patterson et al 1999), responses to zebra mussel infestation (Hallac and Marsden 2000), effects of artificial diets on captive mussels (Nichols and Garling 2002), and seasonal variation in condition (Monroe and Newton 2001).

Traditionally, mussel recapture depends on visual encounters or excavation to locate burrowed mussels. Passive Integrated Transponder (PIT) tags may be an effective tool for tracking translocated mussels to increase accuracy of survival estimates (Kurth et al. 2007, Chapter 2). Using PIT tags enhances mussel recapture at sites where visibility is poor (e.g., turbid water) or when mussels are burrowed in sediments (Kurth et al. 2007, Chapter 2). Reliability of any tagging method depends on tag retention. The tagging

method selected for freshwater mussels depends on shell thickness and the type of habitat into which the tagged mussels will be placed.

Because PIT tags have not been previously used with freshwater mussels, I designed a study to examine whether internal PIT tagging compromises the physiological condition of freshwater mussels. My objectives were to determine if glycogen concentrations in tissue collected from eastern lampmussels (*Lampsilis radiata radiata*) tagged internally with PIT tags differed from glycogen concentrations in non-tagged eastern lampmussels and to determine if there is a relationship between glycogen concentration and mussel size.

Methods

Tissue collection

I collected mantle tissue samples in June 2005 from two groups of eastern lampmussels. The first group comprised 52 eastern lampmussels housed in tanks at the Aquaculture Research Center (ARC) at the University of Maine and used in the internal PIT tagging experiment (Kurth et al. 2007, Chapter 2). I collected samples from 17 control mussels (no PIT tags), 9 mussels with 23 mm tags, 13 mussels with 12 mm tags, and 13 mussels with 12 mm tags with anti-migration caps ~215 days after tagging (~250 days in captivity). I collected samples by inserting a microspreader and micropipette tips between the valves of a slightly gaping mussel and excising a piece of mantle tissue from the mid-ventral region. I placed the tissue into labeled microcentrifuge tubes and placed the tubes on dry ice immediately after collection. I stored the samples at –80^{o} C for 16 m until I measured the glycogen concentrations. Thirty-seven of these mussels (15 control, 7 with 23 mm tags, 7 with 12 mm tags, and 9 with 12 mm tags with caps) were alive to

be relocated to one of three enclosures (1 m x 2 m PVC pipe and rebar frames covered in hardware cloth) in Unity Pond, Maine, in late June 2005.

The second experimental group sampled was comprised of eastern lampmussels (n=85) collected from the Sebasticook River impoundment in June 2005. These animals were divided into 2 internal tagging treatment groups (23-mm tags, *n*= 43; 12-mm tags with an anti-migration cap, *n* = 42) (Kurth et al. 2007, Chapter 2). For a control, I collected mantle tissue from eastern lampmussels (n=27) that had been tagged externally with numbered bee tags and placed in field enclosures in the Sebasticook River in August 2004. Tissue collection and storage methods followed procedures described for mussels retained in the ARC. I maintained the Sebasticook River mussels in the ARC for 21 d to ensure tag retention, and then I placed 1 replicate from each treatment and control group in sand within 3 enclosures in Unity Pond, Maine.

In August 2006, I collected tissue samples from all surviving mussels found in the Unity Pond enclosures, following previously described tissue collection and storage procedures.

Glycogen analysis

Glycogen concentrations were estimated in thawed samples after resuspension in 100mM sodium citrate (Sigma-Aldrich S1804) buffer at a dilution of 1 g tissue to 50 mL buffer (Carr and Neff 1984). I homogenized the samples with a Tissuemiser at maximum speed (30,000 rpm) for a minimum of three 10- second bursts. The homogenate was divided into two aliquots, as was the glycogen standard solution (Sigma-Aldrich G1508 glycogen from *Mytilus edulis,* blue mussel). I used glycogen standard concentrations of 0.6, 0.4, 0.2, and 0.0 mg glycogen/mL buffer solution. One aliquot was digested with

amyloglucosidase (Sigma-Aldrich A7420) (125 µg/mL homogenate), and the other aliquot received an equal amount of buffer. I incubated the aliquots for 2 h at 55°C and then centrifuged the samples at 2000 rpm for 15 minutes. I prepared glucose standards to dilutions of 0.24. 0.16, 0.08, and 0.0 mL glucose/mL deionized water.

I added 10-µL sample/standard to wells of a 96-well round bottom plate. For each sample, I used 2 replicate wells for a digested sample and 2 wells for an undigested sample. For glycogen standards, I used 2 replicate wells for a digested standard dilution and 2 wells for an undigested standard dilution. For the glucose standard, I used 4 wells for each dilution. Glucose in digested and undigested samples (glucose control) was assayed in a spectrophotometer with the glucose oxidase – *o*-dianisidine – peroxidase reaction (Raabo and Terkildsen 1960). The samples were incubated at 37°C for 30 minutes, and the optical density of each sample was read at 450 nm.

Data Analysis

I calculated the standard curve equation for digested and undigested glycogen standards and the glucose standard for each plate. I calculated the trendline equation for digested glycogen by subtracting the undigested standard from the digested. I substracted undigested sample results from digested sample results to get the amount of glycogen digested per sample, and I determined glycogen concentration (mg/mL) for each sample by estimating the amount of glycogen digested from the digested glycogen standard trendline. I determined glycogen concentration of tissue in mg/g:

$$\text{mg glycogen} \cdot \text{g tissue}^{-1} = \frac{G \cdot V}{W}$$

where G = mg/mL glycogen, V = volume (mL) of buffer in homogenate, and W = wet weight (g) of tissue sample. Additionally, I compared pre-tagging glycogen concentrations with mussel length using linear and quantile regression analysis.

Results

Two mussels tagged with 23 mm tags in 2004, over-wintered in the ARC, and moved to the enclosures in June 2005 were resampled in August 2006. One mussel had an initial glycogen level in 2005 (10 m post-tagging) of 2.21 mg/g and gained 0.44 mg/g of glycogen by 2006. The other mussel had an initial glycogen level in 2005 of 3.33mg/g and lost 1.66 mg/g of glycogen by 2006. In August 2006 I resampled 12 control mussels, 34 mussels with 23 mm PIT tags, and 29 mussels with 12 mm tags with caps that were internally tagged in June 2005. Although 13 individuals slightly increased tissue glycogen concentrations from June 2005 to August 2006 (Fig. B.1), there was a net loss of mantle tissue glycogen concentrations across all tagging treatments (Table B.1). Additionally, the initial (2005) glycogen concentrations in the mussels tagged with 23 mm tags was much more variable (range = 0-55.08 mg/g) than those for the 12 mm tag group (range= 0-32.47 mg/g) and the control mussels (range = 0-28.33 mg/g) (Fig. B.1). I did not find a significant relationship between initial glycogen concentrations and mussel length with linear and quantile regression analysis (Fig. B.2).

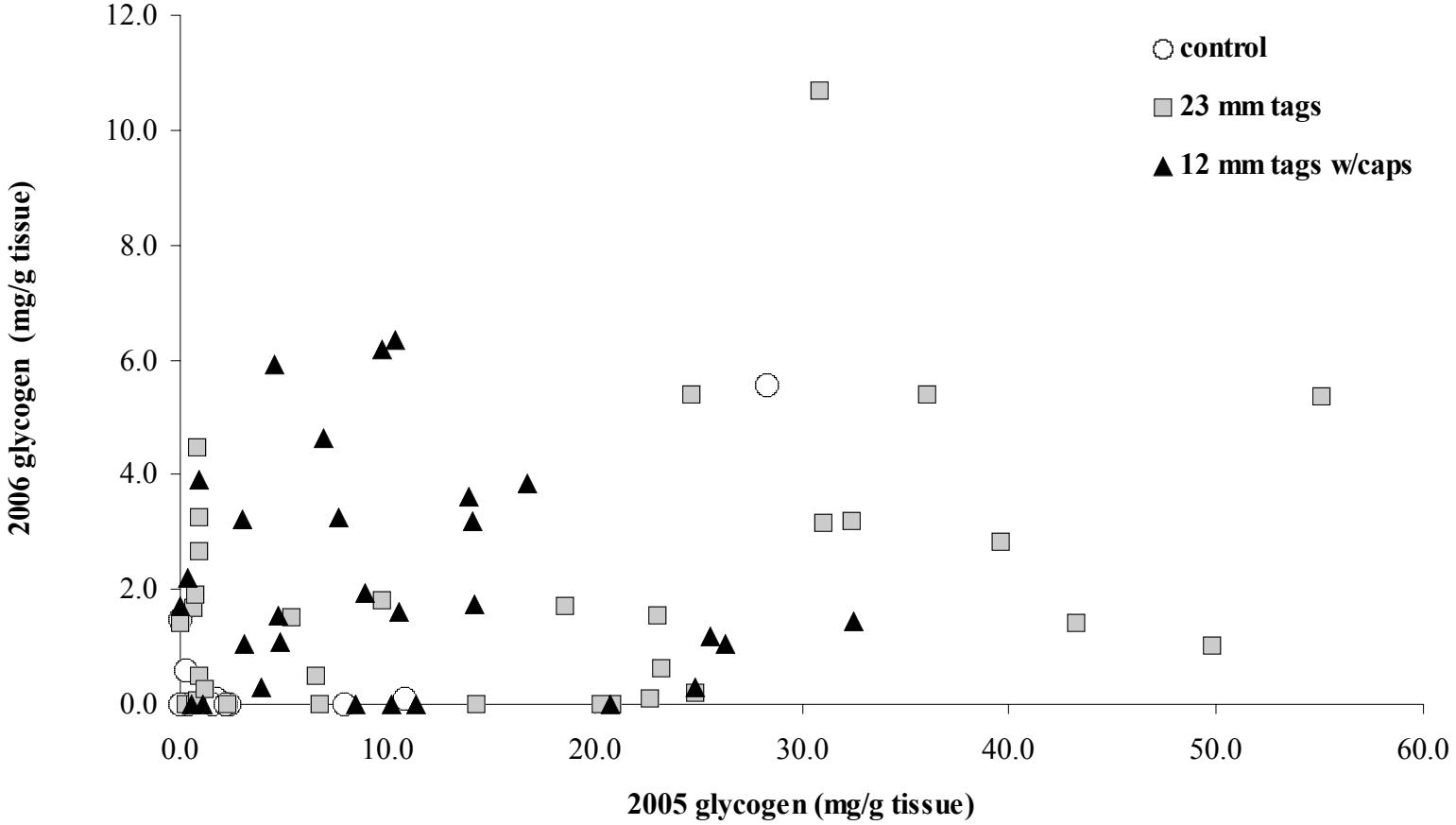

Figure B.1. Glycogen concentrations before PIT tagging (June 2005) versus 14 mo after tagging (August 2006) by treatment in mussels held in enclosures in Unity Pond, Maine.

Table B.1. Means, standard deviations (SD), and change in glycogen concentrations (mg/g tissue) by PIT tag treatment in eastern lampmussels housed in Unity Pond enclosures during June 2005-August 2006.

	Pre-tagging		Post-tagging (14 mo)		Change	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Control (n=12)	4.8	8.12	0.65	1.6	-4.15	6.82
23 mm tags (n=34)	17.56	16.9	1.84	2.31	-15.73	16.35
12 mm tags with caps (n=29)	10.35	8.67	2.11	1.95	-8.24	9.16

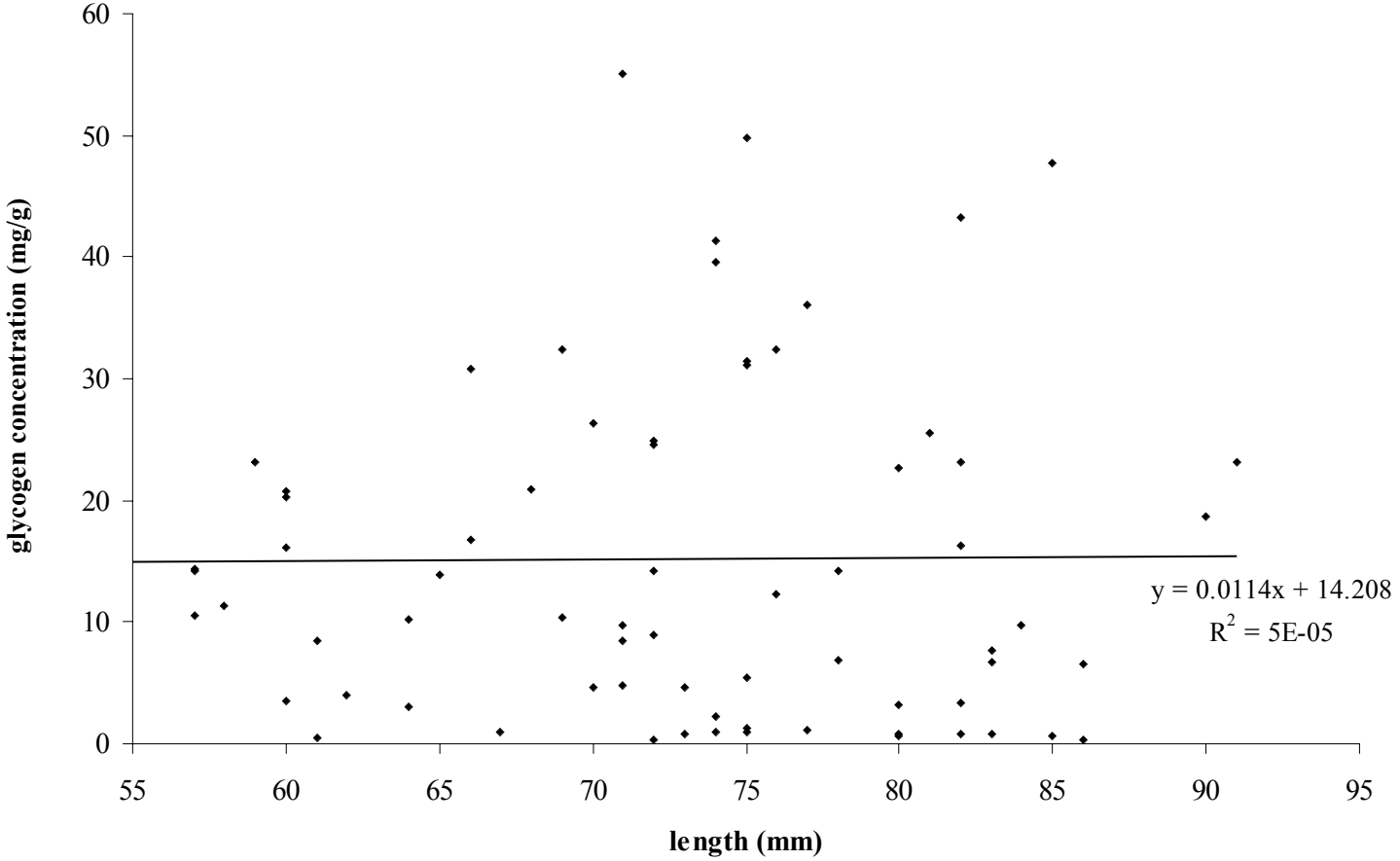

Figure B.2. Linear regression analysis of the relationship between pre-tagging glycogen concentrations (mg/g) and mussel size.

Discussion

I did not find a statistically significant relationship between post-tagging glycogen levels and tagging treatment. Variation in glycogen concentrations indicates that there may be other factors affecting glycogen stores in the study animals that masked tagging effects. Some tagged mussels were gravid when tissue was collected, which may have affected their pre-tagging tissue glycogen levels. Gravid eastern lampmussels may be more susceptible to energetic declines related to environmental stressors due to the high energetic cost of sustaining glochidia in the marsupia throughout the year (Hallac and Marsden 2000). Seasonal glycogen content in mantle tissue of the threeridge mussel (*Amblema plicata*) changed by as much as 72%, which correlated with reproductive activity in that species (Monroe and Newton 2001). Translocating the mussels from the Sebasticook River to Unity Pond may have affected glycogen levels. Handling stress can cause variation in glycogen levels in mussels during the first six months following translocation (Monroe and Newton 2001). Because the mussels for this study were translocated from the Sebasticook River to Unity Pond, there may have been a location effect on all the study animals, regardless of treatment. The control mussels, which would be expected to have the least change in tissue glycogen concentrations, had the lowest glycogen levels at the beginning of the study, and thus they did not serve as true controls. This condition may reflect an effect of being retained in enclosures for 10 months prior to sampling. Ideally, sampling tagged and untagged mussels of the same gender and reproductive status that remain in the Sebasticook impoundment and Unity Pond, concurrently with tagged mussels that are transplanted, would be a better control to test the effect PIT tags on mussel physiological condition.

BIOGRAPHY OF THE AUTHOR

Jennifer E. Kurth was born in Edina, Minnesota. She graduated from John Marshall High School in Rochester, Minnesota, in 1987. She attended the University of Minnesota and graduated in 1998 with a Bachelor of Arts degree in Theatre Arts, and she received a Bachelor of Science degree in 2002 in Biology. She worked as an Academic Advisor at the University of Minnesota for three and a half years before beginning graduate school at the University of Maine. She is a candidate for the Master of Science degree in Ecology and Environmental Science from the University of Maine in May 2007.